T H E S I S

Presented for the Degree of Ph.D.,
Edinburgh University

on

LOUPING-ILL

(An Encephalomyelitis of Sheep)

With Special Reference to Methods of Controlling the Disease

by

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April, 1934.

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INTRODUCTORY

The following thesis had its origin in an investigation into the nature of louping-ill in sheep which the author commenced in 1930 in conjunction with Dr. J. Russell Greig, Messrs. A. Brownlee and D. R. Wilson, and Dr. J. MacLeod.

The scope of the work necessitated the collaboration of a team of workers, each especially concerned with certain aspects of the investigation. The author has been responsible for a study of methods whereby the disease might be controlled, and it is the purpose of this thesis to record the result of original investigations into this aspect of the problem, and to show that prophylactic vaccination of susceptible animals is a practicable method of reducing the mortality.

A SHORT ACCOUNT OF THE NATURE OF LOUPING-ILL IN SHEEP

Louping-ill is responsible for much of the mortality amongst sheep on certain tick-infested hill farms in Scotland and Northern England. Its causation has presented a problem in etiology discussed by various authors for more than a century, and many attempts have been made to ascertain its nature. The history of the disease prior to 1930 has been assembled in a complete review of the literature by POOL (1931) (14,15).

By louping-ill in sheep is currently understood a disease characterised by cerebellar ataxia and disorder of brain and spinal cord functions, lasting about a day in acute infections to some weeks of lingering illness in chronic cases. On farms where the disease is prevalent, sheep with various types of deformity, as a result of paralysis of one or more limbs, are usually encountered. The mortality in definite clinical cases is high, but animals which recover do not usually develop another attack. The association of the disease with particular pastures is well established, and a large proportion of sheep brought from a locality in which louping-ill does not occur into a louping-ill district are liable to become infected. The disease has a seasonal incidence/

incidence which corresponds with the season of maximum activity of ticks, which are habitually present on infected farms. The majority of cases occur in the spring and early summer, and there is a lesser outbreak with smaller losses in the autumn. The death rate varies in different seasons; it is generally high, and in some years it is appalling, as many as thirty or forty per cent. of the lambs being lost. On farms where the disease has existed for a number of years, the mortality is mainly confined to young animals, lambs and hoggs (yearling sheep) being most commonly affected. When, however, the disease makes its appearance on a farm where it has not existed before, animals of all ages are liable to become affected and a heavy mortality generally occurs.

The first conclusive contribution on the causation of this disease was made by POOL, BROWNLEE and WILSON (1930) [16]. These authors recorded the successful transmission of louping-ill in series from sheep to sheep by intracerebral inoculation with material obtained from the central nervous system of affected animals. The pig was also shown to be susceptible. In continuation of the work of Pool, Brownlee and Wilson, the author has been associated in the investigations [2,6,7,8,12,13] which established that:-

(1) the infective agent is a filtrable virus which is communicable to mice as well as to sheep;

(2)/

(2) the pathological changes in the disease are essentially those of an encephalomyelitis;

(3) the virus may be present in an infected sheep without producing typical louping-ill, but blood drawn at an early stage of the febrile reaction, which is a prodromal symptom of this infection, contains the virus;

(4) atypical manifestations may comprise merely a febrile reaction, or the sheep may die without lesions in the brain and spinal cord;

(5) under natural conditions, louping-ill is tick-borne, the vector being Ixodes ricinus L., which is habitually present on louping-ill pastures;

(6) recovery from infection, either naturally or experimentally produced, results in immunity;

(7) the investigation of louping-ill has revealed the presence of another tick-borne infection of sheep. This disease, which has been named "tick-borne fever," is clinically, pathologically, immunologically and etiologically distinguishable from louping-ill, but it probably aggravates the harmful effects of the latter.

That the causal organism of louping-ill is a filtrable virus is now generally accepted (ALSTON & GIBSON)[1] and CZARKOWSKA-GLADNEY & HURST[3]. HURST[9] infected monkeys, and FINDLAY & ELTON[4] transmitted the disease to field voles. There is also unpublished evidence that cattle are susceptible, and may develop the disease naturally. RIVERS & SCHWENTKER[19] record that human beings who have come into close contact with the virus of louping-ill/

ill may develop in their serum neutralising antibodies against the active agent, and illness suggestive of louping-ill infection is reported in three such individuals.

GENERAL TECHNIQUE

Intracerebral Inoculation of Sheep

The operation is performed under general anaesthesia. After surgical preparation of the area of operation, an incision 1 cm. in length is made about 4 cm. behind the horn and about 2 cm. lateral to the median line. A bone drill is inserted in the middle of the incision and the bone perforated to a depth sufficient to permit of the insertion of a small bore hypodermic needle (0.5 mm. diameter). By this means the injection is made directly into the substance of the cerebrum. The dose generally employed is 1.0 c.c.

Intracerebral Inoculation of Mice

The operation is performed under a general anaesthetic (A.C.E. mixture). Injections are made through the skin and bone in the posterior parietal region, at a point slightly lateral to the median line. A 1.0 c.c. tuberculin syringe, fitted with a needle 0.4 mm. in diameter, is suitable for the purpose. The dose generally employed is 0.05 c.c.

Detection/

Detection of Virus in the Blood of Sheep

For this purpose blood is drawn from the jugular vein into a sterile tube containing sufficient potassium citrate to prevent clotting. The citrated blood is diluted to one in five with normal saline, and 0.05 c.c. of this is inoculated intracerebrally into mice. Inoculated mice are kept under observation for a period of not less than twenty days before a negative result is accepted.

Detection of Virus in Tissues

The tissue under examination is pulped in a sterile mortar without sand, and saline is added to make a suspension of 1 in 10. This is allowed to sediment, and 0.05 c.c. of the supernatant fluid inoculated intracerebrally into mice.

INFECTION OF SHEEP BY INTRACEREBRAL INOCULATION WITH LOUPING-ILL VIRUS

Intracerebral inoculation of sheep with louping-ill virus is followed by a febrile reaction which commences on the second or third day after inoculation. During the febrile phase the animal shows symptoms of dullness, followed by symptoms of nervous derangement, which commence about the fifth or sixth day after inoculation. These symptoms coincide with a rapid fall in temperature, and prior to death the temperature/

(1)

Reprinted from "The Journal of Comparative Pathology and Therapeutics."
Vol. XLV. Part 2. pp. 106–140. June, 1932.

Studies in Louping-Ill.

(An encephalomyelitis of Sheep).

I.

Section A.—A Note on the Infectivity of Blood.

Section B.—A Field Experiment (1931), with a preliminary note on the Nature of Tick-borne Fever.

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STUDIES IN LOUPING-ILL.

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I.

Section A : A Note on the Infectivity of Blood.

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INTRODUCTION.

BY louping-ill in sheep is currently understood a disease characterised by cerebellar ataxia and disorder of brain functions, lasting from about a day in acute cases to some weeks in lingering chronic cases. The mortality is high, but *animals which recover do not usually develop another attack.* The association of the disease with particular pastures is well established and sheep brought from a locality in which louping-ill does not occur into a louping-ill district are liable to develop the disease in a large proportion of cases.

In the following paper the evidence will be reviewed showing that, (1) louping-ill is due to a filtrable virus which is communicable to mice as well as sheep ; (2) the virus may be present in an infected sheep without producing typical louping-ill, but blood drawn during the febrile reaction which accompanies this infection contains the virus ; (3) such atypical infections may be merely a febrile reaction or it may be a cause of sudden death in sheep ; (4) under natural conditions louping-ill is probably tick borne ; (5) recovery from infection, either naturally or experimentally produced, results in immunity ; (6) the investigation of louping-ill is rendered difficult by the fact that a distinct type of infection, " tick-borne fever," may co-exist with it. " Tick-borne fever " is clinically and immunologically distinguishable from louping-ill infection, but it probably aggravates the harmful effects of the latter.

The Infective Agent.

A systematic investigation of louping-ill was commenced in 1929 at the Moredun Institute, Animal Diseases Research Association, Edinburgh, and Pool, Brownlee and Wilson (1930), who carried out the investigation, recorded *inter alia* the successful transmission

of the disease in series from sheep to sheep with material obtained from the central nervous system of two sheep affected with louping-ill. No bacterial agent capable of causing the disease was demonstrated, nor did the authors arrive at a definite conclusion as to the nature of the infective agent; they stated, however :—" While filtration experiments have given inconclusive results, none of the work carried out has produced evidence against the existence of an ultra-microscopic virus . . ." and further :—" The type of infection, coupled with the circumstantial evidence that in natural circumstances it is transmitted by ticks, suggests the possibility that it may be associated with Rickettsia."

In continuation of the work of Pool, Brownlee and Wilson, Greig, Brownlee, Wilson and Gordon (1931) repeated the transmission experiments in sheep, established that the infective agent was a filtrable virus, and that it was present in the blood during the febrile reaction. They also showed that sheep which had recovered from the thermal reaction which follows inoculation of virus were immune to subsequent intracerebral inoculation of infective material.

Further progress was facilitated by the observation of Alston and Gibson (1931) that sterile filtrates of material containing the virus of louping-ill reproduced the disease on intracerebral inoculation into mice, and also that the disease could then be transmitted from mouse to mouse indefinitely. The work of Alston and Gibson has been amply confirmed by Czarkowska-Gladney and Hurst (1931) and also in our own experiments, in which several thousand mice have been used.

The symptoms exhibited in the experimentally induced disease in the sheep and mouse indicate involvement of the central nervous system. In the sheep the symptoms are essentially those of a cerebellar ataxia; in the mouse paralysis of one or more limbs usually develops, indicating involvement of the spinal cord.

Brownlee and Wilson (1932) have described the histo-pathology of the naturally occurring disease in the sheep and of the experimentally produced disease in the sheep, mouse and pig. They showed that in the sheep some degree of destruction of the Purkinje cells of the cerebellum occurs, while in the mouse there is necrosis of motor nerve cells of the spinal cord. It was also shown by Hurst (1931) that " . . . intracerebral inoculation of the virus of ' louping-ill ' is productive in both the mouse and the monkey of a definite encephalomyelitis which, while partaking of the general characteristics of neurotropic infections, possesses features peculiar to itself."

The Association of the Tick (Ixodes ricinus) with Louping-Ill.

Circumstantial evidence favours the view that the British tick, *Ixodes rincinus*, *L.* plays an important part in the transmission of louping-ill. Stockman (1918), after completing transmission experiments with ticks, concluded that the highly febrile and sometimes fatal disease induced by the infestation of normal sheep with ticks

which had in their previous stage engorged on affected sheep, was in fact louping-ill. Undoubtedly he produced reactions and sometimes death but the nature of these reactions is a matter for further discussion.

In the paper by Greig *et al.* (1931) experiments designed to test the transmissibility of louping-ill virus by infestation of sheep with ticks are described. In one of the experiments quoted two normal sheep were infested with numerous nymphal and larval ticks gathered from the pastures of a diseased farm during the louping-ill season. Neither of these sheep developed characteristic louping-ill, but on the fourth day after infestation both developed thermal reactions. From one of these sheep blood was drawn during the febrile phase and passed in sheep through two generations by subcutaneous injection; louping-ill did not develop in the inoculated sheep, but a thermal reaction occurred on the fourth day. After recovery from this thermal reaction the sheep were tested for immunity to louping-ill, and all developed a temperature reaction which was followed by typical symptoms of the disease. In discussing these infestation experiments the authors concluded that the first thermal reaction was not due to infection with the virus of louping-ill, as after recovery the sheep were not immune to inoculation with this virus.

There is thus evidence that two sets of workers obtained reactions in sheep by infesting them with ticks collected either from sheep affected with louping-ill (Stockman, 1918), or with ticks collected from diseased farms during the louping-ill season (Greig *et al.*, 1931). While Stockman regarded the reactions as representing louping-ill, Greig *et al.*, in the light of their later acquired knowledge, were able to determine that the febrile affection obtained by them was immunologically distinct from louping-ill.

Immunity.

It is well known that sheep on a diseased farm acquire an immunity to louping-ill, and that it is a highly dangerous practice to introduce sheep from a healthy farm; that sheep on a diseased farm actually acquire immunity has been demonstrated by Pool, Brownlee and Wilson (1930), who inoculated 82 such sheep with louping-ill virus and found that 16 of them were definitely immune.

Immunity can also be induced artificially. Greig *et al.* (1931) found that the introduction of living virus to sheep by intranasal insufflation, intradermal inoculation, or subcutaneous injection, was followed by a thermal reaction and that sheep so treated were immune to subsequent cerebral inoculation.

From a consideration of the literature published up to the present time the following may be concluded :—

(1) The infective agent of louping-ill is a filtrable virus possessing neurotropic characters.

(2) The essential pathology of the disease caused by this virus is represented by a meningo-encephalo-myelitis.

(3) The mouse is susceptible to infection by intracerebral inoculation, and is a suitable test animal for the detection of virus in any given tissue.

(4) Virus can be preserved for long periods in infective sheep brain which has been dried and powdered.

(5) In the sheep a thermal reaction to the introduction of virus is followed by immunity to subsequent intracerebral inoculation of virulent material.

(6) Ticks collected from louping-ill pastures are capable of producing a temperature reaction when allowed to feed on normal sheep. By means of immunity tests this reaction has been proved to be the manifestation of a condition other than that of louping-ill.

(7) Blood drawn during the febrile reaction from a sheep experimentally infected with louping-ill contains the virus.

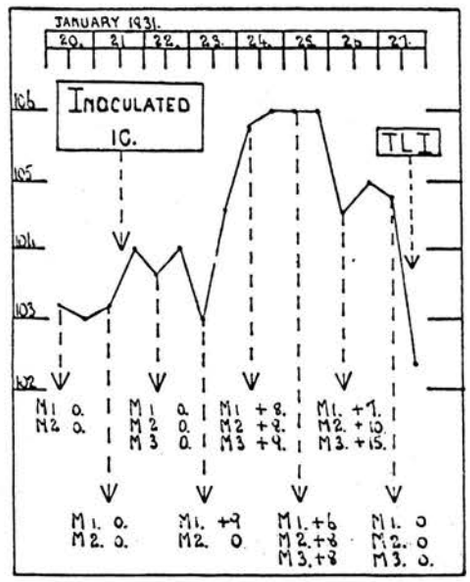
SECTION A.

A Note on the Presence of Virus in the Blood of Infected Sheep.

It has already been demonstrated that the blood of sheep affected with louping-ill may contain living virus (Pool, Brownlee and Wilson, 1930, and Greig *et al.*, 1931). In view of some contemplated experiments with reference to tick transmission of louping-ill, it was decided to obtain an accurate knowledge of the conditions under which virus could be recovered from the blood, and so ascertain when ticks in their various stages of development would suck virus-containing blood from an infected sheep. Our experiments have been made with blood from sheep infected by intracerebral or subcutaneous injection of virulent virus-containing sheep brain, and in some cases with Berkefeld filtrates of such material. Following this injection, samples of blood were collected daily from the jugular vein into sterile tubes containing sufficient potassium citrate to prevent clotting, and mice were used for demonstration of the presence or absence of virus. The citrated blood was diluted to one in five or one in ten with normal saline and 0·1 c.c. of this injected intracerebrally. Inoculated mice were kept under observation for a period of not less than 20 days before a negative result was accepted. We have used a number of sheep for these experiments, and Chart I records in full the result of one such experiment. In this case the sheep was inoculated intracerebrally with a Berkefeld filtrate. A temperature reaction commenced on the second day ; on the fourth day the sheep was dull and showed progressive symptoms of inco-ordination in gait, and by the sixth day the body was in a state of generalised tremor. At this stage the animal was unable to rise and was destroyed. Blood was drawn daily, as indicated by the arrows leaving the underside of the curve, and mice were inoculated intracerebrally with 0·1 c.c. of blood diluted one in ten with saline.

CHART I.

TEMPERATURE CHART OF A SHEEP AFTER INTRACEREBRAL INOCULATION WITH THE VIRUS OF LOUPING-ILL.



M.=Mouse.
O.="No Take."
+X.=Length of Incubation in Days.
TLI. =Typical Louping-ill.

It will be observed that the virus was present in the blood during the course of the disease, appearing on the day on which the temperature rose and persisting until the occurrence of the rapid defervescence before death, at which stage no virus could be detected. In some of our experiments virus in low concentration was shown to be present in the blood up to the time of death of the sheep.

In sheep injected subcutaneously with virulent material there is usually a diphasic temperature curve, and we found on several occasions that during the period of remission between the two phases virus could not be demonstrated in the blood.

From the various experiments carried out, we conclude that louping-ill virus invades the blood stream concurrently with the initial rise in temperature and is present in demonstrable amount during the febrile stage of the infection, and that in most instances a fall in temperature is followed by the disappearance of much or all virus from the blood. These findings are of much value in tick transmission work, as we know now when to feed ticks on presumably infected sheep in order to be sure of their taking in virus.

Section B.

A Field Experiment (1931).

Early in 1931, the Director of the Institute, Dr. J. Russell Greig, arranged for a field experiment in which a group of sheep, rendered immune to louping-ill by the subcutaneous inoculation of living virus, was grazed with a similar number of susceptible sheep on the tick-infested ground of a louping-ill farm. The sheep were transferred early in April to graze on two infected pastures, A and B, each of about 35 acres. They were kept under observation during April, May and part of June—the season during which ticks are most active and during which louping-ill occurs in its maximal incidence. The temperatures of all the sheep on Pasture A were recorded daily, and 15 of the " immunes " and 15 " controls " were weighed twice weekly. The sheep on Pasture B were not handled, but were kept under close observation. It was anticipated that all the sheep would become infested with ticks from these pastures. The time and comparative degree of infestation was recorded by making a daily count of the fully fed nymphal ticks collected from the head, neck and ears of each sheep. This count included only one of the three stages in the life cycle of the tick, but it seemed sufficient to indicate the degree of infestation.

The test sheep were thus exposed to the chance of natural infection and the incidence of louping-ill was observed in each group. On the completion of the field experiment the surviving sheep were returned to the laboratories and were tested for any immunity acquired as a result of exposure to natural infection.

The history of the sheep exposed on Pasture A and those on Pasture B will be dealt with separately, and, in connection with these, Appendices A, B and C have been prepared to give a brief history of each sheep used in the experiment. Appendices A and B refer to the sheep grazed on Pastures A and B respectively, while Appendix C deals with the sheep used as " controls " in the various immunity tests.

Preliminary Experiments in the Immunising of the Sheep used in the Field Experiment.

It was shown by Greig *et al.* (1931) that the subcutaneous inoculation of sheep with living virus was followed by a thermal reaction, and that sheep so treated were immune on recovery. No evidence had been produced to show that any danger attended such a method of treating sheep. From our knowledge of the effect of subcutaneous injection of certain other viruses, we thought it wise to investigate the safety of this method of producing immunity, and to define the dose of living virus which when injected subcutaneously would produce no harmful results and would render the treated sheep immune. Accordingly, twelve sheep were selected for inoculation with living virus. The details of the experiment are shown in Table I.

TABLE I.

Sheep No.	*Subcutaneous inoculation of louping-ill virus* Dose 10 c.c. D.B. 5. Date, 11/2/31.		*Intracerebral inoculation of louping-ill virus.* Dose 1 c.c. 1/100 D.B. 5. Date, 4/3/31
	Dilution.	*Result.*	
994	1/10	Temperature reaction. Sheep slightly dull.	No reaction.
45	1/10	Temperature reaction.	Died at operation.
29	1/100	Temperature reaction.	No reaction.
*42	1/100	No temperature reaction.	No reaction.
996	1/1,000	Temperature reaction.	No reaction.
21	1/1,000	Temperature reaction	No reaction.
986	1/10,000	Temperature reaction. Sheep dull.	No reaction.
987	1/10,000	Temperature reaction.	No reaction.
989	1/100,000	Temperature reaction.	No temperature reaction. Sheep dull.
991	1/100,000	Temperature reaction.	Slight temperature reaction.
20	1/1,000,000	Slight irregularity of temperature.	Temperature reaction.
995	1/1,000,000	Slight irregularity of temperature.	Temperature reaction. T.L.I. March 10, destroyed.
67	—	—	Temperature reaction. T.L.I. March 11, destroyed.

T.L.I.=Typical louping-ill.
D.B.5.=Dried brain strain 1930-1a. (A dried and powdered brain from a louping-ill sheep.) Saline suspensions of this product were used as infective material.
*Sheep 42 was probably immune before treatment.

The result of this titration experiment further substantiated the previous findings that a sheep which gave a decided temperature response to subcutaneous inoculation of virus was solidly immune when subsequently tested by intracerebral inoculation. From this experiment the lowest dose of virus required for the production of solid immunity was taken to be 10 c.c. of 1 in 10,000 *Dried Brain* 5, but as this dose had been decided by the use of two animals only it was considered advisable to use a stronger suspension of virus, and 10 c.c. of a 1 in 1,000 suspension was injected to ensure that the sheep would be definitely immune to intracerebral inoculation of virus.

Sheep Grazed on Pasture A.

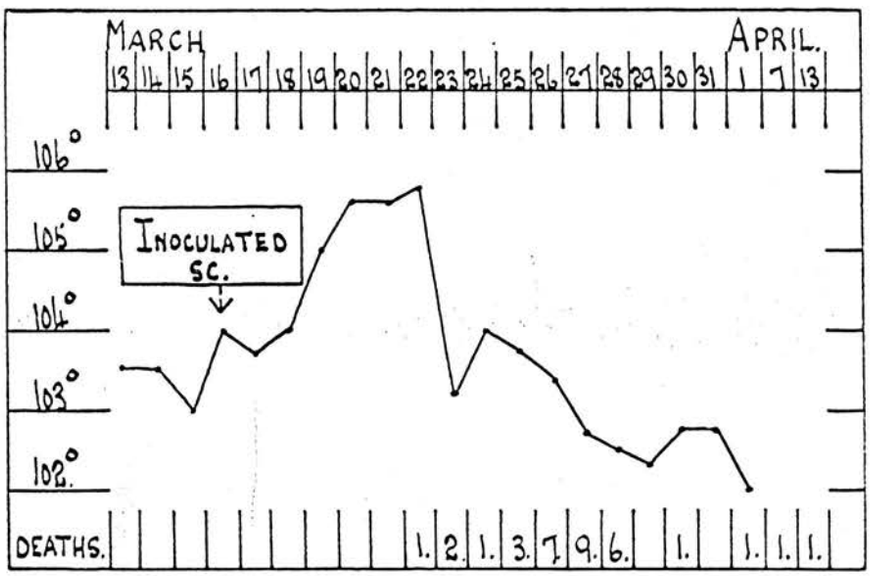
Immunisation.

Fifty sheep were injected on March 16th with 10 c.c. of a 1 in 1,000 saline suspension of *Dried Brain* 5. Their temperatures were recorded daily until April 2nd, when the survivors (with the exception of three—serial Nos. 25, 14 and 33) were transferred to experimental Pasture A. The average daily temperature of the sheep was calculated and from the figures thus obtained the curve in Chart II was prepared. (This chart is referred to as a " composite " temperature chart, and the average rise in temperature is spoken of as a " composite " rise. We have employed this expression in describing the

temperature history of each group of sheep used throughout the experiment.)

CHART II.

COMPOSITE TEMPERATURE CHART OF THE REACTION IN 50 SHEEP AFTER SUBCUTANEOUS INOCULATION WITH THE VIRUS OF LOUPING-ILL.



Thirty-three of the 50 sheep died, and all types of cases developed, including sudden deaths without symptoms having been observed. From this experience it is obvious that living louping-ill virus is unsafe for immunising purposes, as a dose shown to be safe on a small number of sheep may cause disastrous results when used on a moderately large scale.

The 17 surviving sheep (*vide* Appendix A1, serial Nos. 2, 6, 7, 9, 12, 13, 16, 19, 26, 27, 29, 30, 31, 34, 47, 48 and 49), together with seven others (*vide* Appendix A1, serial Nos. 51 to 57), which were already known to be immune, making a total of 24 sheep, formed the protected group for this experiment.

Immediate Results of Exposure to Natural Infection on Pasture A (from April 2nd to June 15th).

To the above 24 immunised sheep were added 25 normal sheep believed to be susceptible to louping-ill as shown by the results of inoculating some of their fellows with living virus ; they comprised the control group (*vide* Appendix A2, serial Nos. 58 to 82).

The temperatures of both groups of sheep were recorded daily while they grazed on the infected pasture, and 15 of the protected sheep, with 15 controls, were weighed twice weekly. Charts III and IV illustrate this phase of the experiment.

CHART III.

COMPOSITE TEMPERATURE CHART OF "IMMUNES" AND "CONTROLS" DURING THE PERIOD OF EXPOSURE TO LOUPING-ILL INFECTION ON A "DISEASED FARM."

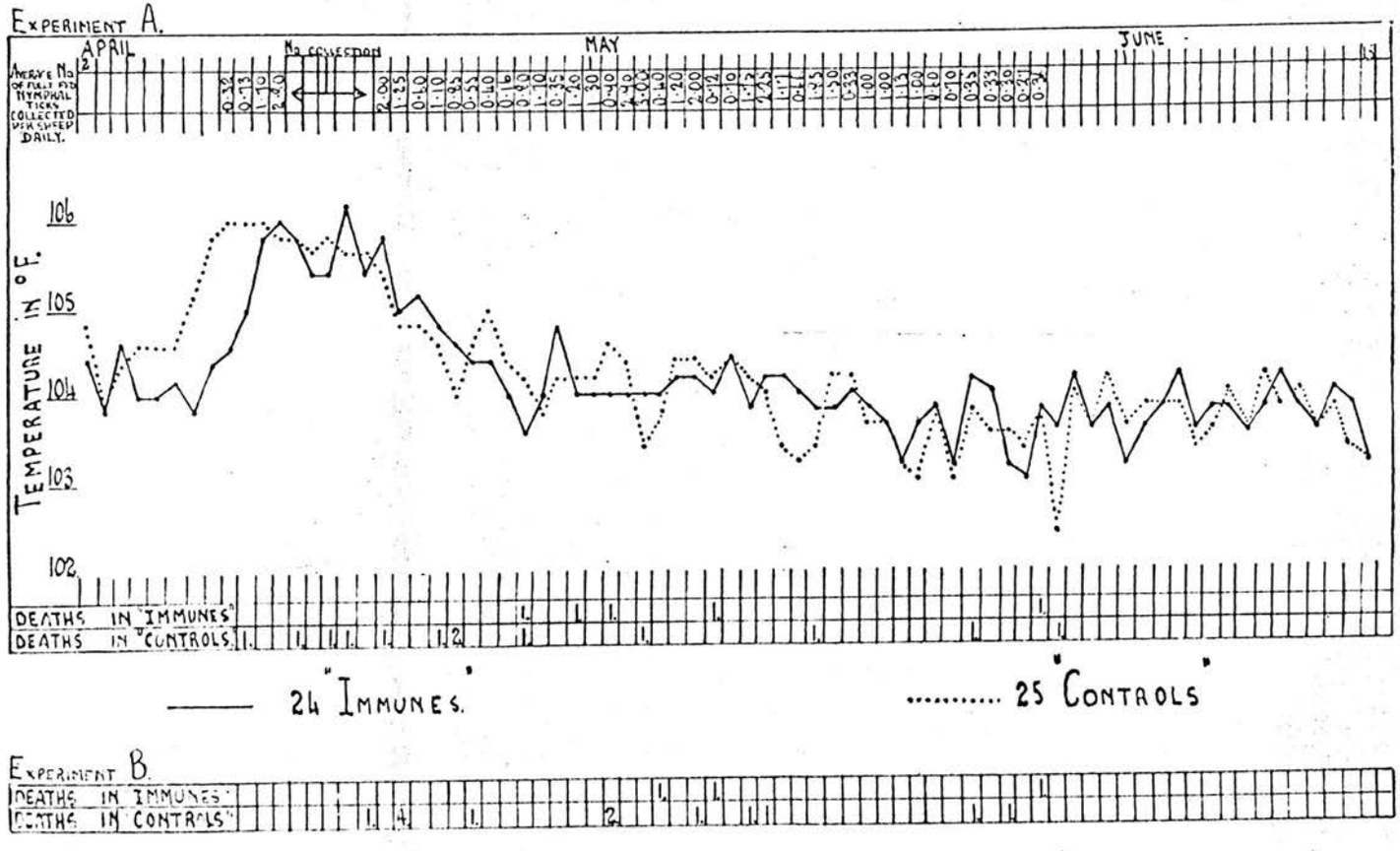
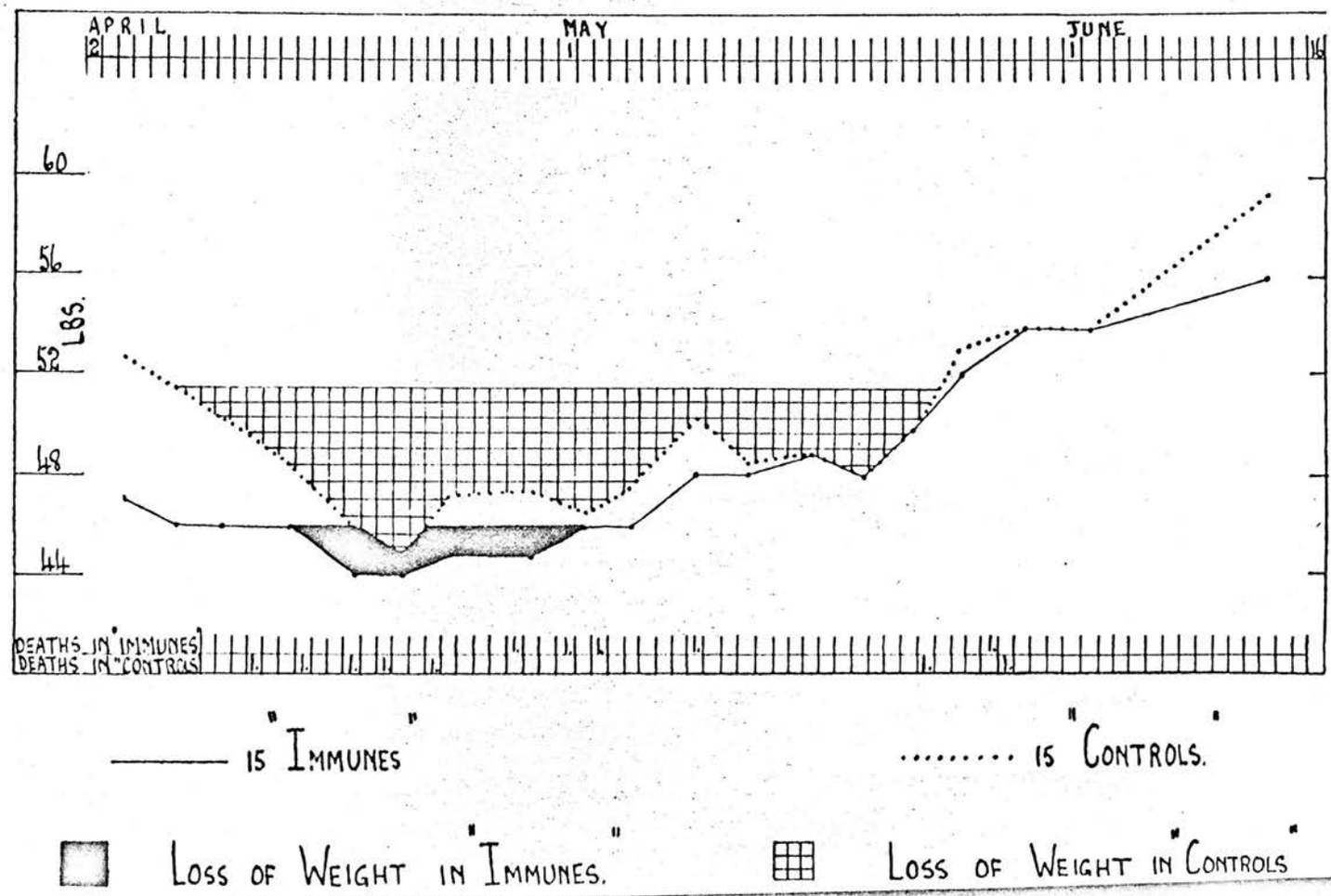


CHART IV.

COMPOSITE CHART OF THE WEIGHT IN LBS. OF 15 "IMMUNES" AND 15 "CONTROLS" DURING THE PERIOD OF EXPOSURE TO "LOUPING-ILL" INFECTION ON A "DISEASED FARM."



The following points are brought out by an examination of these charts :—

1. *Infestation of the Sheep with Ticks.*

The sheep became infested with ticks from the pasture, as indicated by the average daily collection per sheep of fully gorged nymphal ticks. Nymphs replete with blood were collected eight days after the sheep had been put to graze on the experimental pasture. If the " composite " rise in temperature which commenced on the fifth and sixth days was due to an infective agent or agents, transmitted by the tick, then this reaction was produced by a comparatively small number of ticks. It has been shown by one of us (J.M.) that the nymph requires from four to six days to fill itself to repletion with blood. This being so, the ticks collected between April 10th and 13th must have become attached to the sheep between April 4th and 7th. It is hardly conceivable that the light infestation, as judged by these collections, was capable of producing the " composite " rise in temperature by mechanical irritation alone. If the ticks were responsible, it seems feasible to suppose that they were transmitting an infective agent which found in the sheep a suitable host for growth and multiplication.

2. *A "Composite " Rise of Temperature in the "Immunes."*

On the seventh day a rise in temperature commenced in the 24 immunised sheep. By the eleventh day the average temperature was 106° F. and it remained high for eight days. Thereafter it fell gradually until the twenty-second day and remained comparatively steady from this time till the end of the experiment. This temperature reaction, if due to the virus of louping-ill, was quite at variance with our previous finding that after the sheep had given a thermal response to the injection of virus it was subsequently immune. It therefore seemed highly probable that this reaction was not due to the virus of louping-ill. We have since shown that this reaction is due to an infective agent, the nature of which is at present unknown, and which is transmitted by the tick. We have provisionally named this condition " tick-borne fever."

3. *A " Composite " Rise of Temperature in the "Controls."*

On the fifth day a rise in temperature commenced in the 25 control sheep. By the eighth day the average temperature was 106° F. and remained high for nine days. Thereafter it fell gradually until the twenty-first day. Fluctuations in the " composite " temperature occurred until about the forty-third day, and this is the approximate date on which the control sheep commenced to put on weight rapidly.

The foregoing results show that the reaction in the " controls " had a shorter period of incubation than that in the " immunes," which suggests a difference in the nature of the causal factor of the two reactions. As a result of more recent experiments we have

learned that the period of incubation which follows louping-ill infection is shorter than that which follows " tick-borne fever " infection. This knowledge suggests that the early febrile reactions which occurred in the control sheep after exposure to natural infection was caused by the virus of louping-ill, while in the " immune " sheep the longer incubation prior to the febrile reaction was probably a manifestation of " tick-borne fever " infection *per se*.

4. *The Mortality Incidence in the Immune Sheep.*

Five deaths occurred in the 24 immunised sheep during the experiment—a mortality of 20·8 per cent. This was not unexpected, since 17 of these sheep were in a debilitated convalescent state following immunisation, and this condition was further aggravated by the subsequent infection, " tick-borne fever," which was acquired on the experimental pasture. The five deaths were confined to this group of 17 sheep ; none of the seven healthy immune sheep died, although each developed a severe " tick-borne fever " reaction. (For the history of each sheep see Appendix A1 and A2.)

Material for diagnosis was obtained from the five dead sheep (serial Nos. 2, 19, 30, 31 and 47), and the virus of louping-ill was recovered in low concentration from only one of the five, serial No. 47. It is probable that the virus recovered from this sheep had remained alive since immunisation.

5. *The Mortality Incidence in the Control Sheep.*

Of the 25 control sheep, 13 died—a mortality of 52 per cent. No fewer than nine of the thirteen controls which died are accounted for during the " composite " febrile phase, whereas only one of the immunised sheep died during the same period.

The other four sheep (serial Nos. 65, 67, 69 and 73) died of a braxy-like disease ; a bacteriological examination of one of these sheep (serial No. 73) was made, and *Vibrion septique* in pure culture was isolated from the heart blood and peritoneal fluid. The organism was identified morphologically, culturally and serologically.

Four sheep (serial Nos. 61, 66, 78 and 79) died soon after giving birth to dead lambs. Pregnancy was unsuspected, but the births occurred during a febrile reaction in each case, and it is probable that the febrile condition was the actual cause of the premature parturition and the subsequent death of the sheep.

Material for diagnosis was obtained from the central nervous system of five sheep (serial Nos. 60, 69, 71, 75 and 82), and the virus of louping-ill was recovered from two (serial Nos. 71 and 75). Our records show that these were the only sheep which had shown symptoms regarded by us as typical of louping-ill infection.

As a result of subsequent work, it has been found that a sheep may die of louping-ill infection without showing symptoms of involvement of the central nervous system, and in such cases the virus of louping-ill is not present in the central nervous system. The high mortality rate in the " controls " as compared with the

" immunes " would suggest that a number of cases of this type occurred during the course of the field experiment. These acute deaths, although unaccompanied by the usual clinical signs of louping-ill, we regard as really due to the infection.

6. *Loss of Weight in the Immune Sheep and Control Sheep.*

The two " composite " weight curves in Chart IV show a significant difference. A greater loss of weight occurred in the " controls " as compared with the " immunes," and this is further evidence that the " controls " were reacting to a more severe infection than were the " immunes."

Sheep Grazed on Pasture B.

Immunisation.

The death rate in the sheep during the process of immunisation necessitated the preparation of a further group of immune sheep for Pasture B. Accordingly, on March 24th, 1931, ten sheep (serial Nos. 83 to 92) were inoculated subcutaneously with 10 c.c. of 1 in 10,000 *Dried Brain* 5 in saline. On March 25th, 1931, two sheep (serial Nos. 93 and 94) were inoculated subcutaneously with 10 c.c. of 1 in 100,000 *Dried Brain* 5 in saline. On March 26th, 1931, eight sheep (serial Nos. 95 to 102) were inoculated subcutaneously with 10 c.c. of 1 in 10,000 *Dried Brain* 5 in saline.

Of these 20 sheep, four developed symptoms of louping-ill, one recovered, and three died. To the 17 survivors, seven other sheep (serial Nos. 103 to 109), already immune to louping-ill, were added (*vide* Appendix B1). The group of 24 thus formed constituted the immune sheep on Pasture B.

Immediate Results of Exposure to Natural Infection on Pasture B. (*April 2nd and 8th to June 15th.*)

The control group for Pasture B comprised 24 sheep, fellows to the controls on Pasture A (serial Nos. 110 to 133). The history of these sheep appears in Appendix B2. They were kept under close observation, but no temperature records were kept. The dates on which deaths occurred are shown at the bottom of Chart III. The delay in the control death rate in this experiment, as compared with Experiment A, is probably accounted for by the fact that 13 of the control sheep (serial Nos. 110, 111, 113, 114, 119, 120, 121, 124, 127, 128, 130, 131 and 133) were not transferred to the infected pasture until April 8th.

Mortality in the Immunised Sheep.

Three of the 24 immunised sheep died—a mortality of 12·5 per cent. Material for examination was obtained from two of these three sheep (serial Nos. 84 and 91). No virus was recovered from No. 84, but virus in low concentration was recovered from No. 91, and on histological examination there was evidence of slight changes in the central nervous system such as would indicate a recent

reaction to the presence of virus. For several days before it was destroyed this sheep showed symptoms suggestive of a chronic type of louping-ill. We do not hold that this was a breakdown in immunity, but regard the case as most probably a recrudescence of infection, the sheep never having overcome the virus introduced for immunisation. The very severe reactions, followed in some cases by death, which occurred during the immunisation process, support this view. In the case of the third sheep (serial No. 95) pleurisy was the immediate cause of death.

Mortality in the Control Sheep.

Thirteen of the 24 control sheep died—a mortality of 52·4 per cent. Prior to death one of these 13 sheep showed symptoms suggestive of louping-ill infection. Virus was recovered from the central nervous system and other tissues (*vide* Appendix B2, serial No. 113), and the characteristic lesions associated with louping-ill were observed on histological examination.

In six cases (serial Nos. 114, 116, 120, 121, 123 and 159) death was attributed to a braxy-like disease, and a bacteriological examination of one of these (serial No. 114) yielded *Vibrion septique* in pure culture from the stomach wall and heart blood.

Six sheep (serial Nos. 115, 118, 125, 127, 128 and 129) died from undetermined causes. None showed symptoms suggestive of involvement of the central nervous system.

The Testing of Sheep Grazed on Pastures A and B after their Return to the Laboratory (June 15th to August 16th).

Of the 97 sheep grazed on Pastures A and B there were 63 survivors; these were returned to the laboratories on June 15th and comprised 40 " immunes " and 23 " controls."

It was decided to test these sheep for (*a*) immunity to the virus of louping-ill, and (*b*) immunity to the infective agent of " tick-borne fever."

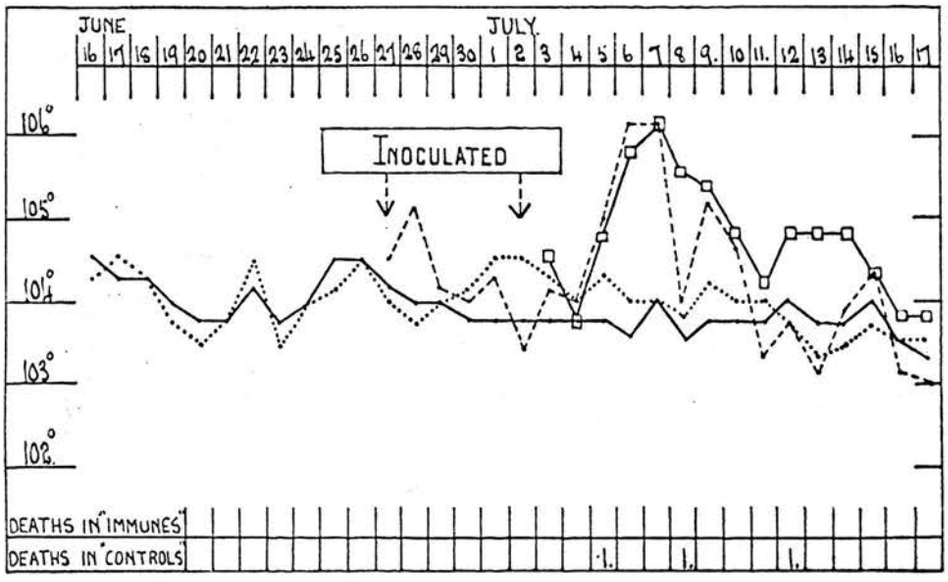
The Test for Immunity to the Virus of Louping-ill.

By inoculation of both groups of sheep with the virus of louping-ill, we hoped to prove that the " immune " sheep still possessed immunity, and to determine if any of the control sheep had developed immunity as a result of their exposure to natural infection. Accordingly, the 63 sheep, with two normal sheep, were inoculated subcutaneously on June 27th with 10 c.c. of 1 in 10,000 *Dried Brain* 5. This dose was decided upon in view of the recognised danger of using a test dose which might prove too severe. Unfortunately on this occasion the two control sheep did not react, and so on July 2nd all 65 sheep, with three other normal sheep, were injected subcutaneously with 10 c.c. of 1 in 1,000 *Dried Brain* 5. Following this inoculation the two original controls reacted, as also did the three additional controls inoculated for the first time on July 2nd.

From this result it was considered that the test dose of virus used was capable of producing a thermal response in sheep not previously immunised. A record of the result is set out in Appendix A3 and B3, and Chart V depicts the result.

CHART V.

COMPOSITE TEMPERATURE CHART OF " IMMUNES " AND " CONTROLS " WHEN TESTED FOR IMMUNITY TO THE VIRUS OF LOUPING-ILL FOLLOWING EXPOSURE TO LOUPING-ILL INFECTION ON A " DISEASED FARM."



——— 40 " Immunes."
. . . . . 23 " Controls."
- - - - 2 Normals to Control the Infectivity of Virus used 27.6.31.
□——□ 3 Normals to Control the Infectivity of Virus used 2.7.31.

When tested subcutaneously with the virus of louping-ill the 40 immunised sheep *ex* Pastures A and B proved immune. Of the 23 control sheep, *ex* Pastures A and B, nine developed a temperature reaction (serial Nos. 59, 63, 76 and 80, *vide* Appendix A3 ; 110, 111, 119, 131 and 132, *vide* Appendix B3), and three of them died. The remaining 14 control sheep (serial Nos. 58, 62, 64, 68, 70, 72, 74 and 81, *vide* Appendix A3 ; 112, 117, 122, 126, 130 and 133, *vide* Appendix B3) did not react to the test dose of virus. That is, 60·9 per cent. of the surviving controls had developed an immunity. It is highly probable that these sheep became immune as a result of infection with virus acquired during the period in which they were exposed to natural infection. The evidence in support of this is contained in Appendix A1 (serial Nos. 1 to 50), which gives the history of 50 sheep of the same group which had never been grazed on infected pasture and which in every case gave a severe temperature

reaction on inoculation with the same virus. If the above recorded percentage (60·9) of the surviving controls had acquired virus while exposed to infection, then it seems highly probable that at least the same percentage of " dead " controls had been infected with virus, and that the deaths in the unprotected sheep from undetermined cause had a louping-ill infection as the primary factor causing death.

Further support for the view that sheep exposed to natural infection may acquire virus without developing typical louping-ill is found in the data set out in Tables II and III, which record the recovery of virus from ticks which fed on a control sheep (serial No. 82, Appendix A2) during the febrile phase prior to death from a braxy-like disease. This sheep developed a temperature reaction which commenced on April 9th ; it was slightly dull on April 13th, and was found dead on April 14th. Post-mortem examination revealed the presence of a necrotic lesion on the mucous membrane of the fourth stomach, which is accepted as diagnostic of " braxy." The virus of louping-ill could not be detected in the central nervous system of this sheep, and on histological examination no lesions of louping-ill could be found. It so happened that ticks replete with blood had been collected from this sheep on April 13th and 14th. In the course of feeding, the ticks had acquired blood during the febrile phase. Fifteen days later these ticks (nymphs and adults) were washed in saline, weighed and ground up in sufficient saline to make a 1 in 10 suspension. This suspension was centrifuged for 15 minutes at about 2,000 R.P.M. From the supernatant fluid progressive dilutions in saline were made to 1 in 10,000. Two mice were inoculated intracerebrally with 0·1 c.c. of each dilution, and kept under observation for 24 days. The result is set out in Table II.

TABLE II.

MICE INOCULATED WITH SALINE SUSPENSIONS OF EMULSIFIED TICKS FED ON SHEEP (SERIAL NO. 82).

Mice inoculated ic.	*Result.*		
2 M. 1 in 10	+ 9	+ 18	T.L.I.
2 M. 1 in 100	0	0	
2 M. 1 in 1,000	0	0	
2 M. 1 in 10,000	0	0	

+x = Death x days after inoculation.
0 = " No take."
M. = Mouse.
T.L.I. = Typical louping-ill.

The two mice inoculated from the 1 in 10 suspension developed symptoms typical of louping-ill infection. Virus was recovered from the central nervous system of these mice, and passed in series through mice and sheep, as indicated in Table III.

TABLE III.

RECORD OF THE INFECTIONS RESULTING FROM THE INOCULATION OF BRAIN SUSPENSION PREPARED FROM THE TWO MICE WHICH DIED FOLLOWING INOCULATION OF EMULSIFIED TICKS.

2 M. 1 in 10

+ 9 T.L.I.		+ 18 T.L.I.	
3 M. ++ + 6 T.L.I.		2 M. + + 5 T.L.I.	
S. 224 + T.L.I.	2 M. ++ 6 T.L.I.	2 M. ++ 5 T.L.I.	S.232 + T.L.I.
2 M. 1/10	++ 6 T.L.I.	2 M. 1/100	+ 8 + 9 T.L.I.
2 M. 1/100	++ 6 T.L.I.	2 M. 1/1,000	++ 9 T.L.I.
2 M. 1/1,000	++ 6 T.L.I.	2 M. 1/10,000	0 + 14 T.L.I.
2 M. 1/10,000	++ 6-7 T.L.I.	2 M. 1/100,000	0 0

M. = Mouse.
S. = Sheep.
O. = "No take."
+x = Death x days after inoculation.
T.L.I. = Typical louping-ill.

THE TEST FOR IMMUNITY TO "TICK-BORNE FEVER."

The Nature of "Tick-borne Fever."

We have already indicated that the temperature reaction in the "immune" sheep grazed on Pasture A was caused not by the louping-ill virus, but by an infective agent or agents transmitted by ticks, and that the reaction in the control sheep so grazed might be due to the presence of the true louping-ill virus in the blood stream, or to the agent responsible for the reaction in the "immunes," or to both. Also, as already described, there was some evidence suggesting that the reactions in the two groups of sheep were not identical.

For purposes of description, we have named the reaction which occurred in the "immune" sheep "reaction of immunes," and that which occurred in the "control" sheep "reaction of controls."

Blood drawn from an "immune" sheep (serial No. 56) on April 15th, when its temperature was 107·8° F., and from a "control" sheep (serial No. 79) on April 15th, when its temperature was 107·4° F., was shown to contain no louping-ill virus, by intracerebral inoculation of sheep. By the subcutaneous inoculation of sheep with these specimens of blood we have subsequently passed the causal agent of both reactions through many generations—26 in the case of the "immune," and 27 in the case of the "control." In each series there has been produced a disease characterised by a low mortality, with an incubation period of about four days, followed by a sharp rise in temperature and a period of fever, which is often irregular and very prolonged but usually lasts about nine days.

We have now shown by transmission experiments that this reaction is a "tick-borne fever." Ticks collected from the pasture,

and ticks collected from reacting sheep, have produced a temperature reaction when allowed to feed on normal sheep. The reaction can be produced at will with ticks known to be infected, and it has been proved to be similar in its manifestation to that which occurred in the " immune " sheep while these were grazing on tick-infested ground.

" Tick-borne fever " can be readily distinguished from louping-ill by the following facts :—

(*a*) The incubation period of " tick-borne fever " is longer than that of louping-ill.

(*b*) The two diseases are immunologically distinct.

(*c*) Louping-ill mortality is high, " tick-borne fever " mortality is low.

(*d*) Mice can be infected with louping-ill but not with " tick-borne fever."

(*e*) In louping-ill infection there is an encephalomyelitis. In " tick-borne fever " infection encephalomyelitis is not present.

We believe " tick-borne fever " to be due to a specific infective agent transmitted by the tick, but the nature of the infective agent has not yet been determined. Filtration experiments have, so far, yielded negative results, and examinations for the presence of bacteria and protozoon parasites have also been attended with negative results. The type of the disease, coupled with the evidence that it is transmitted by ticks, suggests the possibility that the infective agent may be Rickettsia. This aspect of the problem is the subject of further work, and will be dealt with in future publications.

Results of Testing for Immunity to " Tick-borne Fever."

Sixty sheep (40 " immunes " and 20 " controls "), returned to the laboratory from Pastures A and B, were tested for immunity to " tick-borne fever." Blood from immune and control sheep, referred to above, and proved to contain the infective agent of " tick-borne fever " in each case, was used as follows :—

Nineteen " immunes " *ex* Pasture A inoculated with 5 c.c. of " control reactor's " blood.

Twenty-one " immunes " *ex* Pasture B inoculated with 5 c.c. of " immune reactor's " blood.

Ten " controls " *ex* Pasture B inoculated with 5 c.c. of " control reactor's " blood.

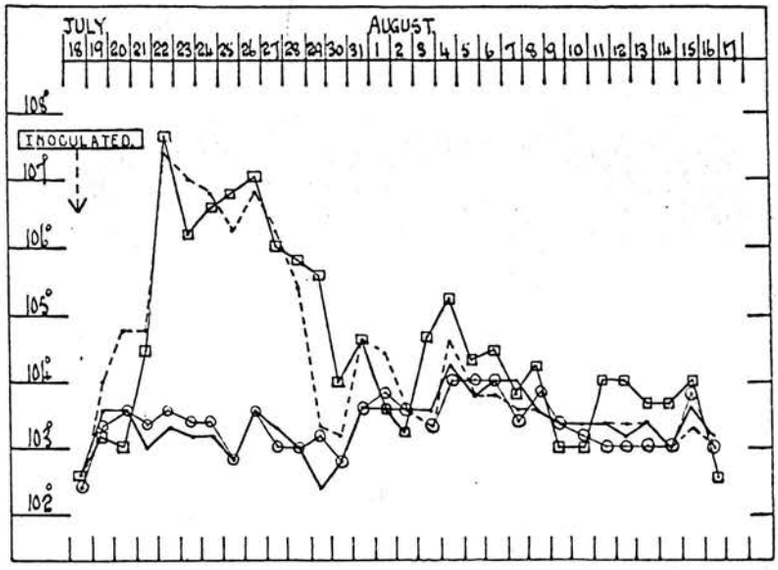
Ten " controls " *ex* Pasture A inoculated with 5 c.c. of " immune reactor's " blood.

We thus tested 21 louping-ill immune sheep with " immune reactor's " blood and 19 with " control reactor's " blood, also ten control sheep with " immune reactor's " blood, and ten control sheep with " control reactor's " blood.

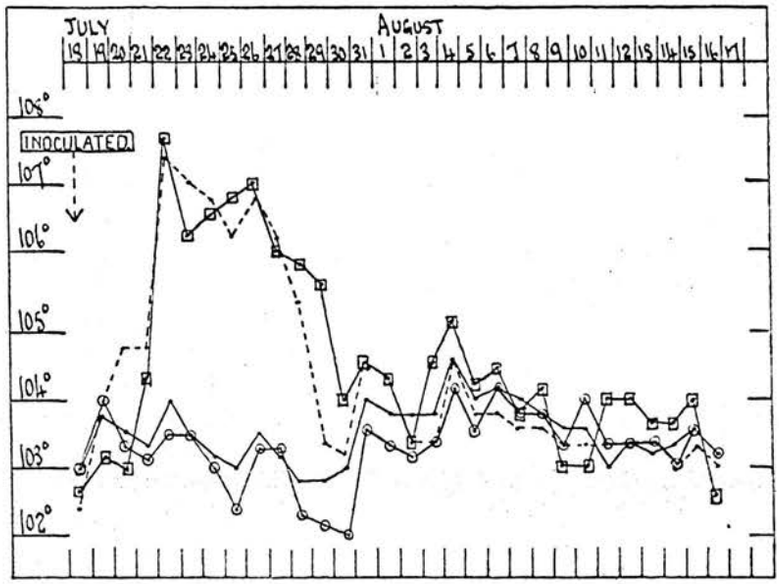
The result of these tests is shown in Appendices A3 and B3. The reactions which occurred in the eight sheep used to control the infectivity of the blood are described in Appendices C2 and C3, and the results are depicted in Charts VI and VII.

CHARTS VI AND VII.

COMPOSITE TEMPERATURE CHART OF "IMMUNES" AND "CONTROLS" WHEN TESTED FOR IMMUNITY TO TICK BORNE FEVER FOLLOWING EXPOSURE TO LOUPING ILL INFECTION ON A "DISEASED FARM."



——— 19 "Immunes" Ex Pasture A. Tested with "C.R." Blood.
- - - - 4 Normal Sheep to Control the Infectivity of the "C.R." Blood.
○——○ 21 "Immunes" ex Pasture B. Tested with "I.R." Blood.
□——□ 4 Normal Sheep to Control the Infectivity of the "I.R." Blood.



——— 10 "Controls" ex Pasture B. Tested with "C.R." Blood.
- - - - 4 Normal Sheep to Control the Infectivity of the "C.R." Blood.
○——○ 10 "Controls" ex Pasture A Tested with "I.R." Blood.
□——□ 4 Normal Sheep to Control the Infectivity of the "I.R." Blood.

For abbreviations see p. 140.

All the sheep, with the exception of one (serial No. 110), after return from the field experiment were immune when tested with these two types of infective blood, whereas sheep which had not been on the experimental farm gave a marked thermal response. In other words, the temperature reaction which occurred in both the immune and control sheep while on the experimental pasture, and which has been subsequently proved to be a " tick-borne fever," was followed by an immunity to the inoculation of blood known to be infective. The only death which occurred (serial No. 110) was inexplicable.

Discussion.

A field experiment is described in which a group of 48 sheep rendered immune to the virus of louping-ill, together with a group of 49 susceptible sheep (controls), were grazed on a pasture where louping-ill was known to occur. During the period of exposure to infection the temperatures of 24 sheep in each group were recorded daily ; also 15 of the " immune " and 15 " controls " were weighed twice weekly. All the sheep became infested with ticks, and in every sheep (immune and control) of which a temperature record was kept a febrile reaction developed.

It has been shown that the reaction in the " immune " sheep is attributable to an infective agent transmitted by the ticks, and the condition has been provisionally named " tick-borne fever."

The cause of the reaction in the " control " sheep was attributable, in at least a large proportion of cases, to the infective agent of " tick-borne fever," together with the virus of louping-ill ; this conclusion is supported by the following facts :—

(*a*) A greater loss of weight occurred in the " controls " as compared with that in the " immunes."

(*b*) The febrile affection in the "controls " had a shorter period of incubation than that in the " immunes."

(*c*) 60·9 per cent. of the surviving " controls " had acquired immunity to the virus of louping-ill, and all had acquired immunity to " tick-borne fever."

(*d*) The presence of virus in the blood of a sheep prior to death from a braxy-like disease has been demonstrated. This sheep showed no symptoms of louping-ill and virus could not be demonstrated in the central nervous system, and no lesions of louping-ill were present.

The following is the analysis of the mortality incidence in the immunised and control sheep.

14	Healthy immune sheep :	mortality, nil.	... ...	0	per cent.
34	Convalescent immune sheep :	mortality, 8	... ...	23·5	„
Total 48	Immunised sheep :	mortality, 8	... ...	16·6	„
49	Control sheep :	mortality, 26	... ...	53·0	„

The mortality incidence in the 48 immunised sheep is accounted for by the fact that many of them were in a debilitated convalescent state following immunisation with living virus, and that death occurred

in eight of them was not unexpected. The remaining 14 immunised sheep had completely recovered after immunisation and, although each developed a febrile reaction indicative of " tick-borne fever " infection, none of these sheep died.

We did not examine the tissues of all control sheep which died because many showed no clinical evidence of central nervous system infection which we believed at that time to be the *only* characteristic of louping-ill. We now know that the virus of louping-ill may cause the death of sheep which present no symptoms of central nervous system infection. Accordingly, the fact that many of the surviving control sheep had acquired immunity to louping-ill indicates that a natural infection with the virus had occurred during the period of exposure on the infected pasture, and it is probable that many of the control sheep which died were primarily infected with the virus of louping-ill and succumbed to this infection.

These findings suggest that many sheep on a louping-ill farm acquire virus during the louping-ill season. Some develop a mild reaction, recover, and are subsequently immune. Some develop symptoms indicative of infection of the central nervous system, and it has been our experience that in these cases lesions of louping-ill can be demonstrated in, and virus recovered from, the brain and spinal cord. Our experience, however, also indicates that in many other cases no symptoms such as have been previously associated with louping-ill develop, and the sheep are found dead from undetermined cause.

On many louping-ill farms " tick-borne fever " probably occurs in individual sheep concurrently with louping-ill infection, and this fact may account for some of the difficulties experienced by previous investigators in establishing the etiology of louping-ill. From the point of view of immunisation against louping-ill, it is fortunate that the mortality incidence following " tick-borne fever " infection *per se* is low. The significance, if any, of the association of the two diseases is not clear, and is the subject of further study. As a result of subsequent work, it has been established that the majority of the ticks collected from this experimental pasture harboured the infective agent of " tick-borne fever " while only a few have been proved to harbour the virus of louping-ill.

In consideration of the heavy mortality that occurred in the control sheep, and of the fact that a number of the survivors had acquired immunity to louping-ill, it is possible that the process of " acclimatisation " of the sheep on many of the hill farms in Scotland represents in reality the development of immunity to the virus of louping-ill, but during the process of " acclimatisation " a heavy annual toll on the sheep population of " diseased " farms is exacted. If this immunity can be established by reliable artificial methods, it is probable that louping-ill will be brought under effective control. The part which " tick-borne fever " infection may play in " acclimatisation " has yet to be determined.

Conclusions.

1. It may now be taken as proved that louping-ill is due to a distinct filter passing virus.
2. The clinical manifestations of typical louping-ill are due to infection of the central nervous system with this virus.
3. The virus has been detected in the blood in cases in which symptoms of louping-ill did not subsequently develop. The symptoms of infection in such cases is a febrile reaction occasionally followed by death.
4. The above data suggest that for the occurrence of typical louping-ill invasion of the central nervous system by the virus is essential. It would appear that accessory conditions favour such invasion, *e.g.* the occurrence of tick-borne fever. (See No. 7.)
5. Recovery from the infection, either naturally or experimentally produced, results in immunity to louping-ill.
6. The evidence so far indicates that the use of a living virus for prophylactic immunisation is not a safe procedure.
7. As a result of the experimental investigation of louping-ill it has been shown that ticks (*Ixodes ricinus*) which transmit the virus also harbour another infective agent which causes febrile disease in sheep. Cross immunity experiments have shown that this disease is distinct from louping-ill. The nature of the causal agent is so far unknown.

The Appendix.

The abbreviations used in this appendix and elsewhere in this report are shown on page 140.

There are three appendices: A, B and C. Appendices A and B follow the same sequence as the text of the paper.

Appendix A deals with all sheep grazed on Pasture A and is divided into three phases.

Each sheep has been given a serial number and by following the serial number of any surviving sheep its history can be traced through each phase.

Appendix B is compiled on the same lines as "Appendix A," and deals with the sheep grazed on Pasture B.

Appendix C.

I. The history of five normal sheep acting as controls to prove the infectivity of virus used for testing the "Immune" and "Control" sheep grazed on Pastures A and B for immunity to "louping-ill."

II. The history of four normal sheep acting as controls to prove the infectivity of the "Control reactors" blood, which was used for testing the "Immune" sheep grazed on Pasture A and the "Control" sheep grazed on Pasture B.

III. The history of four normal sheep acting as controls to prove the infectivity of the "Immune reactors" blood, which was used for testing the "Immune" sheep grazed on Pasture B and the "Control" sheep grazed on Pasture A.

APPENDIX A I.

THE HISTORY OF THE SHEEP DURING IMMUNISATION FOR PASTURE A.

Ser. No.	*S. No.*	*Result of subcutaneous inoculation with 10 c.c. D.B. 5. Date 16/3/31.*	
1	80	T.R., T.L.I.	+ 10
2	81	T.R., V.D.	L
3	82	T.R.	+ 12
4	83	T.R., T.L.I., 9th, 10th day.	+ 11
5	84	T.R., T.L.I., 9th, 10th day.	+ 11
6	85	T.R.	L
7	86	T.R.	L
8	87	T.R., T.L.I., 9th day.	+ 10
9	88	T.R., V.D.	L
10	89	T.R., T.L.I., 12th day.	+ 12
11	90	T.R., T.L.I., 9th day.	+ 10
12	91	T.R., D.	L
13	92	T.R., D.	L
14	93	T.R., V.D., 16th to 21st day.	+ 22
15	94	T.R., T.L.I., 11th day.	+ 12
16	95	T.R.	L
17	96	T.R., T.L.I., 8th, 9th day.	+ 10
18	97	T.R., T.L.I., 10th day.	+ 11
19	98	T.R., V.D.	L
20	99	T.R., T.L.I., 12th day.	+ 12
21	100	T.R., T.L.I., 11th day.	+ 11
22	101	T.R., T.L.I., 10th day.	+ 11
23	102	T.R., T.L.I., 8th day.	+ 9
24	103	T.R., T.L.I., 8th day.	+ 8
25	104	T.R., V.D.	+ 17
26	105	T.R., D.	L
27	106	T.R., D.	L
28	107	T.R., T.L.I., 10th day.	+ 11
29	108	T.R., D.	L
30	109	S.T.R., D.	L
31	110	T.R., V.D.	L
31	111	T.R., T.L.I., 10th day.	+ 11
33	112	T.R., V.D.	+ 28
34	113	T.R.	L
35	114	T.R., T.L.I., 10th day.	+ 11
36	115	T.R., T.L.I., 5th, 6th day.	+ 7
37	116	T.R., T.L.I., 10th day.	+ 10
38	117	T.R., T.L.I., 9th day.	+ 10
39	118	T.R., T.L.I., 8th day.	+ 9
40	119	T.R., T.L.I., 12th day.	+ 12
41	120	T.R., T.L.I., 5th, 6th day.	+ 7
42	121	T.R., T.L.I., 6th day.	+ 6
43	122	T.R., T.L.I., 9th day.	+ 50
44	123	T.R., T.L.I., 10th, 11th day.	+ 12
45	124	T.R.	+ 11
46	125	T.R., T.L.I., 9th day.	+ 9
47	126	T.R., V.D.	L
48	127	T.R., D.	L
49	128	T.R.	L
50	129	T.R., T.L.I., 12th, 13th day.	+ 14

Ser. No.	*S. No.*	*Date.*	*Inoculum*	*Result.*	
51	20	Feb. 11	10 c.c. 1/1,000,000 D.B.5. sc.	N.T.R.	
		Mar. 4	1 c.c. 1/100 D.B.5. ic.	T.R.	L
52	21	Feb. 11	10 c.c. 1/1,000 D.B. 5. sc.	T.R.	
		Mar. 4	1 c.c. 1/100 D.B.5. ic.	N.T.R.	L
53	29	Feb. 11	10 c.c. 1/100 D.B.5. sc.	T.R., D.	
		Mar. 4	1 c.c. 1/100 D.B.5. ic.	N.T.R.	L
54	986	Feb. 11	10 c.c. 1/10,000 D.B.5. sc.	T.R., D.	
		Mar. 4	1 c.c. 1/100 D.B.5. ic.	N.T.R.	L
55	989	Feb. 11	10 c.c. 1/100,000 D.B.5. sc.	T.R.	
		Mar. 4	1 c.c. 1/100 D.B.5. ic.	N.T.R., D.	L
56	991	Feb. 11	10 c.c. 1/100,000 D.B.5. sc.	T.R.	
		Mar. 4	1 c.c. 1/100 D.B.5. ic.	N.T.R.	L
57	994	Feb. 11	10 c.c. 1/10 D.B.5. sc.	T.R., S.D.	
		Mar. 4	1 c.c. 1/100 D.B.5. ic.	N.T.R.	L

APPENDIX A II.

THE HISTORY OF THE "IMMUNES" WHILE EXPOSED TO INFECTION ON PASTURE A. PERIOD APRIL 2nd TO JUNE 15th.

	Ser. No.	*S. No.*	*Date T. rose.*	*Date and height of first peak.*	*T. in ° F. fluctuated between.*	*T. normal.*	*Symptoms. Post-mortem. Material examined.*	*Result.*
Convalescent Immune Sheep.	2	81	Ap. 10	13th 105·4	101·8-105·8	—	Ap. 17-29 weak and dull. Ap. 30 died. Cerebrum, cerebellum. cervical, dorsal and lumbar cords for diagnosis. No virus recovered. Slight infiltration into meninges over cerebellum.	+28
	6	85	Ap. 11	13th 107	103-106·4	May 22	—	L
	7	86	Ap. 8	9th 107·6	102·6-107	May 13	—	L
	9	88	Ap. 10	11th 106·4	102-107·4	May 28	—	L
	12	91	Ap. 10	12th 107·2	103-106	May 7	—	L
	13	92	Ap. 9	10th 106·4	102-107	May 27	—	L
	16	95	Ap. 9	14th 107·2	102-107·2	May 16	—	L
	19	98	Ap. 11	12th 107·2	103-106·8	Ap. 27	May 22-26 very weak and dull. May 27 dead. Cerebrum, cervical and lumbar cord for diagnosis. No virus recovered. No lesions found.	+55
	26	105	Ap. 8	11th 107	102-107·2	May 16	—	L
	27	106	Ap. 14	16th 107·6	102·4-106	May 2	—	L
	29	108	Ap. 10	13th 107·2	103-107·2	May 5	—	L
	30	109	Ap. 13	15th 106·6	102·8-107·2	Ap. 20	May 6 T. fell to 101° F. May 6-7 dull and weak. May 8 dead. Cerebrum, cerebellum, cervical, dorsal and lumbar cords for diagnosis. No virus recovered.	+36
	31	110	Ap. 8	12th 107	104-106	—	T. remained high till Ap. 20. Fell gradually to 95·6 on Ap. 27. Ap. 20-26 weak. Ap. 27 dead. Cerebrum, cerebelum, cervical dorsal and lumbar cords for diagnosis. No virus recovered.	+25
	34	113	Ap. 10	13th 106	102·6-106·4	May 4	—	L
	47	126	Ap. 12	13th 106	103·2-105·6	Ap. 18	Ap. 18-May 1 dull and weak. May 2 found dead. Cerebrum, cerebellum, cervical, dorsal and lumbar cords for diagnosis. Virus recovered from 1 out of 6 mice inoculated.	+30
Healthy Immune Sheep.	48	127	Ap. 10	11th 107	103-107·8	June 1	—	L
	49	128	Ap. 19	21st 107·8	102-106	May 18	—	L
	51	20	Ap. 11	13th 107·4	104-107·6	June 4	—	L
	52	21	Ap. 11	14th 107·6	104-107·8	May 8	—	L
	53	29	Ap. 8	10th 108	103-107·6	May 23	—	L
	54	986	Ap. 11	12th 107·4	104-108	Ap. 28	—	L
	55	989	Ap. 11	12th 107·6	102-107	May 19	—	L
	56	991	Ap. 9	13th 108	103·2-108·2	May 14	—	L
	57	994	Ap. 7	10th 106·8	102-107·6	May 4	—	L

APPENDIX A II (*Continued*).

THE HISTORY OF THE "CONTROLS" WHILE EXPOSED TO INFECTION ON PASTURE A. PERIOD APRIL 2nd TO JUNE 15th.

Ser. No.	*S. No.*	*Date T. rose.*	*Date and height of first peak.*	*T. in ° F. fluctuated between.*	*T. normal.*	*Symptoms. Post-mortem. Material examined.*	*Result.*
58	130	Ap. 8	12th 107·4	102-107·6	May 14	Ap. 21-25 dull and trembling.	L
59	132	Ap. 9	11th 106·4	104-107·4	May 4	Ap. 12 slightly dull.	L
60	133	Ap. 9	11th 106·6	101·4-107·4	Ap. 30	Ap. 10-May 28 S.D. and weak. May 28 T. fell to 99° F. Found dead same day. Cerebrum, cervical and lumbar cords for diagnosis. No virus recovered. No lesions found.	+56
61	134	Ap. 7	9th 106·8	103-106	Ap. 18	Ap. 13 dead lamb born. Ap. 14-22 dull and weak. Ap. 23 found dead.	+21
62	135	Ap. 6	9th 107	102·6-107	May 10	—	L
63	138	Ap. 4	8th 107·2	102-107·8	May 18	—	L
64	139	Ap. 10	11th 107·4	101·6-107·4	May 16	—	L
65	142	Ap. 7	8th 106·8	105·6-106·8	Ap. 20	2nd rise in T. Ap. May 1 105·6° F., May 2 105·4° F., May 3 106° F., May 4 found dead. Appearance of anærobic infection of abdominal cavity. Braxy type of case.	+32
66	143	Ap. 7	8th 107	104·8-106·8	—	T. remained high till Ap. 16 when dead lamb was born. Ap. 17 T. fell to 101·4° F. Found dead same day.	+15
67	145	Ap. 15	17th 106·6	102·6-108	—	T. fell from 106° F. on Ap. 25 to 98·4° F. on Ap. 26 when sheep was lying on its side unable to rise. Ap. 27 found dead. Acute enteritis.	+25
68	149	Ap. 7	9th 107	104·4-107	Ap. 18	—	L
69	150	Ap. 7	9th 106·8	—	—	Ap. 10 105·6° F. found dead. Braxy type of case. Cerebrum, cerebellum, cervical, dorsal and lumbar cords for diagnosis. No virus recovered. No lesions found.	+ 9
70	152	Ap. 16	17th 107·6	100·8-106·6	May 15	May 10—June 15 very weak.	L

Ser. No.	*S. No.*	*Date rose.*	*T.*	*Date and height of first peak.*	*T. in ° F. fluctuated between.*	*T. normal*	*Symptoms. Post-mortem. Material examined.*	*Result.*
71	153	Ap.	7	8th 107·2	103-106·4	—	May 12, 13, 14 very dull, straddling and irregular gait. Found stretched out showing occasional convulsions. May 14 destroyed. Cerebrum, cerebellum, cervical, dorsal and lumbar cords for diagnosis. Virus recovered highly infective dilution 1 in 10,000. Lesions of L.I. in C.N.S.	+42
72	158	Ap.	8	13th 107·6	102·6-107	May 26	—	L
73	160	Ap.	8	10th 106·8	105·2-107	—	T. fell gradually to 102·4° F. on Ap. 22. Dull and weak. Found dead. Braxy-like disease. *Vibrion septique* recovered from H.B. and peritoneal fluid. Identified morphologically, culturally and serologically.	+20
74	164	Ap.	8	11th 106·8	104·4-107	May 24	—	L
75	167	Ap.	8	10th 107	105-107·4	—	T. high till Ap. 21 sudden fall to 100. 6° F. on Ap. 23. Lying on its side unable to rise. Padling movements of limbs. Slight salivation. Tremors of lips. Destroyed. Virus recovered from cerebrum, cerebellum, hippocampus, spinal ganglia, cervical, dorsal and lumbar cords, sciatic nerve, pancreas and adrenal gland. Highest infective dilution, cerebellum 1 in 100,000. Lesions of L.I. throughout the C.N.S.	+21
76	168	Ap.	11	12th 107	102-107·4	May 24	—	L
77	169	Ap.	7	10th 107·4	103·8-106·8	May 4	2nd T.R. from May 13-23 100·8, 102·2 105·8, 106·6, 103·8, 102·4, 102·2, 102·2, 101·6, 100·4, 100. During this period very dull and weak. May 23 found dead. Carcase œdematous.	+51

Ser. No.	*S. No.*	*Date T. rose.*	*Date and height of first peak.*	*T. in ° F. fluctuated between.*	*T. normal.*	*Symptoms. Post-mortem. Material examined.*	*Result.*
78	171	Ap. 8	10th 106·8	104·8-105·8	—	Dead lamb born Ap. 16, found dead same day.	+14
79	173	Ap. 11	12th 106·4	—	—	T. reached its height Ap. 15 107·4, 16 107, 17 104·6, 18 98 when sheep lambed. Ap. 19 found dead.	+17
80	175	Ap. 8	9th 106·8	102·2-107	June 4	—	L
81	177	Ap. 7	12th 107·6	102·4-107·4	May 24	Ap. 12-19 dull.	L
82	178	Ap. 9	10th 107	—	—	Ap. 11 T. 107, 12 106, 13 104·6 when sheep was slightly dull. Ap. 14 found dead. Braxy type of case. Necrotic area in the fourth stomach. Cerebrum, cerebellum cervical, dorsal and lumbar cords for diagnosis. No virus recovered. No lesions found.	+12

APPENDIX A III.

PERIOD JUNE 16th TO AUGUST 16th.

The history of the " immunes " *ex* Pasture A when tested for :—

1.—Immunity to " louping-ill."
2.—Immunity to " tick-borne fever."

Ser. No.	*S. No.*	1.—" *Louping-ill* " *Immunity Test.* *Result of two inoculations with D.B. 5. sc.—one on 27/6/31, the other on 2/7/31.*	2.—" *Tick-borne fever* " *Immunity Test.* *Result of sc. inoculation with 5 c.c. C.R. blood on 18/7/31.*
6	85	N.T.R.	N.T.R.
7	86	N.T.R.	N.T.R.
9	88	N.T.R.	N.T.R.
12	91	N.T.R.	N.T.R.
13	92	N.T.R.	N.T.R.
16	95	N.T.R.	N.T.R.
26	105	N.T.R.	N.T.R.
27	106	N.T.R.	N.T.R.
29	108	N.T.R.	N.T.R.
34	113	N.T.R.	N.T.R.
48	127	N.T.R.	N.T.R.
49	128	N.T.R.	N.T.R.
51	20	N.T.R.	N.T.R.
52	21	N.T.R.	N.T.R.
53	29	N.T.R.	N.T.R.
54	986	N.T.R.	N.T.R.
55	989	N.T.R.	N.T.R.
56	991	N.T.R.	N.T.R.
57	994	N.T.R.	N.T.R.

PERIOD JUNE 16th TO AUGUST 16th.

The history of the " controls " *ex* Pasture A when tested for :—

1.—Immunity to " louping-ill."

2.—Immunity to " tick-borne fever."

Ser. No.	*S. No.*	1.—" *Louping-ill* " *Immunity Test.* *Result of two inoculations with D.B. 5 sc.—one on 27/6/31, the other on 2/7/31.*	2.—" *Tick-bourne fever.*" *Immunity Test.* *Result of sc. inoculation with 5 c.c. I.R. blood on 18/7/31.*
58	130	N.T.R.	N.T.R.
59	132	T.R. July 8 died. No virus recovered.	
62	135	N.T.R.	N.T.R.
63	138	T.R.	N.T.R.
64	139	N.T.R.	N.T.R.
68	149	N.T.R.	N.T.R.
70	152	N.T.R. Very weak and dull.	N.T.R. June 29 sheep in debilitated condition. Destroyed.
72	158	N.T.R.	N.T.R.
74	164	N.T.R.	N.T.R.
76	168	T.R. Symptoms of T.L.I. July 11. July 13 destroyed. No virus recovered from C.N.S. Lesions of L.I. in cervical and lumbar cords.	
80	175	T.R. June 29-July 9. July 4 sheep very dull.	N.T.R.
81	177	N.T.R.	N.T.R

APPENDIX B I.

THE HISTORY OF THE SHEEP DURING IMMUNISATION FOR PASTURE B.

Ser. No.	*S. No.*	*Date of Injection.*	*Inoculum*	*Result.*	
83	68	24/3/31	10 c.c. 1/10,000 D.B. 5. sc.	N.T.R.	L
84	62	"	"	N.T.R.	L
85	63	"	"	N.T.R.	L
86	66	"	"	T.R.	L
87	72	"	"	T.R.	L
88	73	"	"	T.R., T.L.I. 8th–9th day	L
89	74	"	"	T.R.	L
90	76	"	"	T.R.	L
91	78	"	"	T.R.	L
92	69	"	"	T.R., T.L.I. 9th day	+10
93	65	25/3/31	10 c.c. 1/100,000 D.B. 5. sc.	N.T.R.	L
94	70	"	"	N.T.R.	L
95	997	26/3/31	10 c.c.1/10,000 D.B. 5. sc.	T.R., S.D.	L
96	990	"	"	T.R., D.	L
97	75	"	"	T.R.	L
98	988	"	"	T.R., D.	L
99	983	"	"	T.R.	L
100	981	"	"	T.R., V.D.	L
101	64	"	"	T.R., T.L.I. 7th day	+ 8
102	993	"	"	T.R., T.L.I. 10-12th day	+13
103	71	11/3/31	Suspension of mouse brain strain ewe 5 1929 1 c.c.ic.	T.R., V.D. Slight tremors of head March 18.	L
104	996	11/2/31	10 c.c. 1/1,000 D.B. 5 sc.	T.R.	
		4/3/31	1 c.c. 1/100 D.B. 5. ic.	N.T.R.	L
105	42	11/2/31	10 c.c. 1/100 D.B. 5. sc.	V.S.T.R.	
		4/3/31	1 c.c. 1/100 D.B. 5. ic.	N.T.R.	L
106	987	11/2/31	10 c.c. 1/10,000 D.B. 5. sc.	V.S.T.R.	
		4/3/31	1 c.c. 1/100 D.B. 5. ic.	S.D.	L
107	*36	8/1/31	20 c.c. L.I. Vaccine sc.	N.T.R.	
		22/1/31	20 c.c. L.I. Vaccine sc.	N.T.R.	
		5/2/31	1 c.c. 1/100 D.B. 5. ic.	N.T.R.	
		4/3/31	15 c.c. 1/10 D.B. 12. I.M.	N.T.R.	L
108	37	8/1/31	10 c.c. 1/100 D.B. 5. sc.	T.R., T.L.I. 11-14 day.	
		5/2/31	1 c.c. 1/100 D.B. 5. ic.	N.T.R.	
		4/3/31	15 c.c. 1/10 D.B. 12. I.M.	N.T.R.	L
109	44	22/1/31	10 c.c. 1/100 D.B. 5. sc.	T.R., S.D.	
		5/2/31	1 c.c. 1/100 D.B. 5. ic.	N.T.R.	
		4/3/31	15 c.c. 1/10 D.B. 12. I.M.	N.T.R.	L

* The vaccine used for immunising this sheep was an experimental product the nature of which will be described in a later publication.

APPENDIX B II.

THE HISTORY OF THE "IMMUNES" WHILE EXPOSED TO INFECTION ON PASTURE B.

PERIOD APRIL 2nd TO JUNE 15th.

	Ser. No.	*S. No.*	*Symptoms.*	*Post-mortem.*	*Material examined.*	*Result.*
Convalescent Immune Sheep	83	68		—		L
	84	62	May 13-27 dull, chronic diarrhœa. May 27 found dead.		Cerebrum, cervical, dorsal and lumbar cords for diagnosis	+55
					No virus recovered. No lesions found.	
	85	63		—		L
	86	*66		—		L
	87	*72		—		L
	88	*73		—		L
	89	*74		—		L
	90	*76		—		L
	91	*78	May 2 lame. May 3 unable to rise. May 5-7 paddling movements of limbs. May 8 destroyed.			+30
					Virus recovered from 1 of 2 mice inoculated.	
					Mild lesions of louping-ill in C.N.S.	
	93	65		—		L
	94	70		—		L
	95	*997	May 2 very lame. Disinclined to move. May 5 found dead.			+27
					P.M. septic pleurisy.	
	96	*990		—		L
	97	*75	May 19-20 sheep dull. June 9-10 very dull.			L
	98	*988		—		L
	99	*983		—		L
	100	*981		—		L
Healthy Immune Sheep	103	71		—		L
	104	996		—		L
	105	42		—		L
	106	987		—		L
	107	*36		—		L
	108	*37		—		L
	109	*44		—		L

THE HISTORY OF THE "CONTROLS" WHILE EXPOSED TO INFECTION ON PASTURE B.

PERIOD APRIL 2nd TO JUNE 15th.

Ser. No.	*S No.*	*Symptoms.*	*Post-mortem.*	*Material examined.*	*Result.*
110	*131	Ap. 28-May 13 slightly dull.	Chronic diarrhœa.		L
111	*136		—		L
112	137		—		L
113	*140	May 9 lying alone. Inco-ordination of gait. Occasional periods of stupor. May 11 found dead. On the ground there was evidence that paddling movements of the limbs had occurred.		Virus recovered from cerebrum, cerebellum, hippocampus, spinal ganglia, cervical, dorsal and lumbar cords, sciatic and median nerves, pancreas and adrenal gland. Highest infective dilution cerebellum 1 in 100. Lesions of louping-ill throughout C.N.S.	+33
114	*141	May 7 found dead.	P.M. appearance suggestive of anærobic infection of abdominal cavity. Braxy type of case.	*Vibrion septique* recovered from H.B. and stomach wall. Identified morphologically, culturally and serologically.	+29
115	144	May 9 dull. May 10 found dead.	No definite cause of death.		+38
116	146	Ap. 20 just able to stand. Found dead same day.	P.M. appearance suggestive of anærobic infection of abdominal cavity. Braxy type of case.		+18
117	147		—		L
118	148	Ap. 29-May 2 weak. May 2 found dead.	P.M. organs anæmic.		+30
119	*151		—		L
120	*154	Ap. 20 found dead.	P.M. appearance suggestive of anærobic infection of abdominal cavity. Braxy type of case.		+12
121	*155	Ap. 29 diarrhœa. May 2 found dead.	P.M. appearance suggestive of anærobic infection of abdominal cavity. Braxy type of case.		+24
122	156		—		L
123	157	Ap. 24 found dead.	P.M. suggestive of anærobic infection of abdominal cavity. Braxy type of case.		+22
124	*159	Ap. 18 found dead.	P.M. appearance suggestive of anærobic infection of abdominal cavity. Braxy type of case.		+10
125	161	Ap. 20 found dead.	P.M. organs anæmic. Slight parasitic infestation of lungs.		+18
126	162		—		L
127	*163	Ap. 29-May 25 weak. May 25 found dead.	P.M. organs anæmic.		+47
128	*165	Ap. 20 found dead.	P.M. organs anæmic.		+12
129	166	May 21-23 dull. May 23 found dead.	P.M. organs anæmic. Excess of fluid in serous cavities.		+51
130	*170		—		L
131	*172		—		L
132	174	May 2 dull.			L
133	*176		—		L

* Sheep which were not put on to the pasture until Ap. 8. The others were put on the pasture on Ap. 2.

APPENDIX B III.

PERIOD JUNE 16th TO AUGUST 16th.

The history of the " immunes " *ex* Pasture B when tested for :—

1.—Immunity to " louping-ill."

2.—Immunity to " tick-borne fever."

Ser. No.	*S. No.*	1.—" *Louping-ill* " *Immunity Test.* *Result of two inoculations with D.B.5. sc.—one on 27/6/31, the other on 2/7/31.*	2.—" *Tick-borne fever* " *Immunity Test.* *Result of sc. inoclation with 5 c.c. I.R. blood on 18/7/31.*
83	68	N.T.R.	N.T.R.
85	63	T.R. Not considered to be due to the virus. Quite atypical, occurred 14 days after inoculation.	N.T.R.
86	66	N.T.R.	N.T.R.
87	72	N.T.R.	N.T.R.
88	73	N.T.R.	N.T.R.
89	74	N.T.R.	N.T.R.
90	76	N.T.R.	N.T.R.
93	65	N.T.R.	N.T.R.
94	70	N.T.R.	N.T.R.
96	990	N.T.R.	N.T.R.
97	75	N.T.R.	N.T.R.
98	988	N.T.R.	N.T.R.
99	983	N.T.R.	N.T.R.
100	981	N.T.R.	N.T.R.
103	71	N.T.R.	N.T.R.
104	996	N.T.R.	N.T.R.
105	42	N.T.R.	N.T.R.
106	987	N.T.R.	N.T.R.
107	36	N.T.R.	N.T.R.
108	37	N.T.R.	N.T.R.
109	44	N.T.R.	N.T.R.

PERIOD JUNE 16th TO AUGUST 16th.

The history of the " controls " *ex* Pasture B when tested for :—

1.—Immunity to " louping-ill."

2.—Immunity to " tick-borne fever."

Ser. No.	*S. No.*	1.—" *Louping-ill* " *Immunity Test.* *Result of two inoculations with D.B. 5. sc.—one on 27/6/31, the other on 2/7/31.*	2.—" *Tick-borne fever* " *Immunity Test.* *Result of sc. inoculation with 5 c.c. C.R. blood on 18/7/31.*
110	131	T.R. commenced June 29. Fluctuated between 103-106·6. July 12 normal.	July 21 T.R. commenced. July 22 106·4, 23 105, when sheep died. No virus recovered. No lesions found.
111	136	July 1 T.R. commenced. 2 107·4, 3 106·4, 4 103·8, very dull, head handing. July 5 found dead. No virus recovered.	
112	137	N.T.R.	N.T.R.
117	147	N.T.R.	N.T.R.
119	151	June 29 T.R. commenced. July 2 106·2. Fluctuated between 103·4-105·8 till July 14	N.T.R.
122	156	N.T.R.	N.T.R.
126	162	N.T.R.	N.T.R.
130	170	N.T.R.	N.T.R.
131	172	July 4 T.R. commenced July 7 105·8. Fluctuated between 104·2-105·8 till July 14.	N.T.R.
132	174	T. rose to 106·4 on July 5.	N.T.R.
133	176	N.T.R.	N.T.R.

APPENDIX C I.

THE HISTORY OF FIVE NORMAL SHEEP TO CONTROL THE INFECTIVITY OF THE VIRUS OF LOUPING-ILL USED WHEN TESTING THE IMMUNITY OF THE "IMMUNES" AND "CONTROLS" *ex* PASTURES A AND B.

Ser. No.	*S. No.*	*Result of two inoculations with D.B. 5. sc., one on 27/6/31, the other on 2/7/31.*
134	230	T.R. commenced July 5. July 6 106·4. Fluctuated between 104-107·2 till July 13 when it returned to normal.
135	223	T.R. commenced July 4. July 5 106. July 6 106. July 7 106·4. July 8 normal.
136	219	T.R. commenced July 4. July 7 107·4. Fluctuated between 103·8-106·2 till July 15 when it returned to normal.
137	196	T.R. commenced July 4. July 7 106·2. Remained between 104·2-106 till July 18 when it returned to normal.
138	194	T.R. commenced July 4. July 6 106·4. Fluctuated between 103·6-106 till July 12 when it returned to normal.

APPENDIX C II.

THE HISTORY OF FOUR CONTROL SHEEP TO PROVE THE INFECTIVITY OF THE "CONTROL REACTORS" BLOOD USED FOR TESTING THE "IMMUNES" *ex* PASTURE A AND THE "CONTROLS" *ex* PASTURE B. SHEEP INOCULATED SC. WITH 5 C.C. OF "I.R." BLOOD ON 18/7/31.

Ser. No.	*S. No.*	*T.R. commenced.*	*Date and height of first peak.*	*T. in ° F. fluctuated between.*	*T. returned to normal.*
*134	230	July 21	July 22 106·8	102·4-107·2	August 16
*135	223	July 20	July 22 107·6	102-107·4	August 9
139	218	July 20	July 22 108·4	103-106·4	August 16
140	1	July 21	July 22 107·6	103·107·6	August 1

APPENDIX C III.

THE HISTORY OF FOUR CONTROL SHEEP TO PROVE THE INFECTIVITY OF THE "IMMUNE REACTORS" BLOOD USED FOR TESTING THE "IMMUNES" *ex* PASTURE A AND THE "CONTROLS" *ex* PASTURE B. SHEEP INOCULATED SC. WITH 5 C.C. OF "C.R." BLOOD ON 18/7/31.

Ser. No.	*S. No.*	*T.R. commenced.*	*Date and height of first peak.*	*T. in ° F. fluctuated between.*	*T. returned to normal.*
*136	219	July 21	July 22 107	103-106·8	August 1
*137	196	July 21	July 22 108	102·4-107·6	August 2
141	202	July 19	July 20 106·4	102·8-107·8	August 1
142	197	July 21	July 22 107·6	105·6-107	July 29

* Had already been used as controls to prove the infectivity of the virus of "louping-ill." (*Vide* appendix C I.)

ABBREVIATIONS.

S.	=	Sheep.
Ser.	=	Serial.
ic.	=	Intracerebral inoculation.
sc.	=	Subcutaneous inoculation.
im.	=	Intramuscular inoculation.
D.B.5.	=	Dried brain 5. A dried and powdered infective brain. Strain of virus Case I 1930 (Greig *et al.* 1931.)
T.	=	Temperature.
N.T.R.	=	No temperature reaction.
V.S.T.R.	=	Very slight temperature reaction.
S.T.R.	=	Slight temperature reaction.
T.R.	=	Temperature reaction.
S.D.	=	Slightly dull.
D.	=	Dull.
V.D.	=	Very dull.
T.L.I.	=	Typical louping-ill.
+X	=	Death x days after inoculation or after being put on to infected pasture.
O	=	No take.
L	=	Lived.
P.M.	=	Post-mortem examination.
C.N.S.	=	Central nervous system.
H.B.	=	Heart blood.
I.R.	=	Immune reactors blood, *i.e.*, infective blood obtained from a louping-ill immune sheep while exposed to infection on infected pasture.
C.R.	=	Control reactors blood, *i.e.*, infective blood obtained from a control sheep while exposed to infection on infected pasture.

ACKNOWLEDGMENTS.

To our Director, Dr. J. Russell Greig, we wish to extend our grateful thanks for his constructive criticism and advice.

To Mr. Andrew Linton, B.SC., of Gilmanscleuch, Ettrick, we are especially indebted for the full facilities in field investigation which he has afforded us.

We also desire to record our appreciation of the enthusiastic support, and capable assistance, which has been rendered by the staff of this Institute. In this connection, we would particularly mention the name of Mr. G. H. Annat, for his valuable services in the field.

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temperature in some cases may be subnormal. Louping-ill virus can be detected in the blood concurrently with the initial rise in temperature, and is present in demonstrable amount during the febrile stage of the infection. In most instances, a fall in temperature is followed by the disappearance of much or all virus from the blood. Fig. I, taken from a paper by Gordon, Brownlee, Wilson & MacLeod (reprint enclosed: "Studies in Louping-ill. I.") depicts the result of such an experiment.

It will be observed that the virus of louping-ill was present in the blood on the day on which the temperature commenced to rise, and persisted until the occurrence of the rapid defervescence before death, at which stage virus was not detected in the blood.

INFECTION OF SHEEP BY SUBCUTANEOUS INOCULATION WITH LOUPING-ILL VIRUS

Subcutaneous inoculation of sheep with louping-ill virus is generally followed by a diphasic temperature reaction. In some cases symptoms indicative of central nervous system involvement develop and the animal dies. In rare instances these symptoms appear and pass off, and the animal makes a complete recovery. In many cases, however, the only manifestation of infection is a febrile reaction during which virus can be detected in the blood. Following a reaction of either of the last two types, the recovered animals are always immune to infection by intracerebral inoculation. Fig. II depicts a typical/

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"Tick-Borne Fever"

(A HITHERTO UNDESCRIBED DISEASE OF SHEEP)

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Reprinted from

THE JOURNAL OF COMPARATIVE PATHOLOGY AND THERAPEUTICS

Vol. XLV. Part 4. pp. 301-312. December, 1932.

"TICK-BORNE FEVER"

(A Hitherto Undescribed Disease of Sheep.)

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IN the course of preliminary studies on the tick transmission of louping-ill, MacLeod (1932) showed that ticks collected in louping-ill districts were capable of setting up a febrile reaction in sheep. He also showed that animals which had recovered from this reaction were not immune to louping-ill.

In a previous paper (Gordon *et al.*, 1932) we described a field experiment in which 25 sheep immune to louping-ill were grazed with 24 non-immune "control" sheep on the tick-infested pasture of a louping-ill farm. While the sheep were on this pasture their temperatures were recorded daily. In the initial stages of louping-ill infection a temperature reaction occurs, and it was expected that such reaction would be observed in the "controls," while in the immune animals no rise in temperature would take place. As already recorded, however, in every sheep, both immune and control, a febrile reaction developed. This unexpected reaction in sheep believed to be immune to louping-ill has been the subject of further investigation which is here described, and which shows that the condition was a febrile tick-borne affection identical with that observed by MacLeod (1932). This condition we have, therefore, named "tick-borne fever." Detailed experiments recording the successful transmission of this disease by the nymphal and adult stages of the sheep tick, *Ixodes ricinus*, are described by MacLeod and Gordon (1932, a).

In this paper we record the result of our preliminary observations on the nature of the disease, and describe experiments to show that (1) the febrile reactions which occurred in louping-ill immune and louping-ill non-immune sheep while grazed on a "diseased farm" were due to "tick-borne fever" infection, (2) a specific immunity to this disease can be produced in sheep, and (3) louping-ill and "tick-borne fever" are immunologically distinct.

The Nature of the Disease.

The disease is characterised in its natural incidence by an incubation period of varying length, usually between four and eight days.

followed by a febrile phase which lasts about ten days, though it is often irregular and very prolonged. During the febrile period, the affected sheep appears dull and listless, and there is often a considerable loss of body weight. If the disease be uncomplicated by other infections recovery usually occurs, though a small percentage of affected animals die. In sheep killed during the febrile phase enlargement of the spleen has been the most constant macroscopic lesion found, and in a limited number of histological examinations of the spleen, mesenteric glands and central nervous system no definite pathological changes have been observed. The infection can be passed from sheep to sheep in series by the subcutaneous inoculation of blood from affected animals, and the incubation period following such inoculation is usually four days. The nature of the infective agent has not yet been established notwithstanding many attempts to demonstrate the presence of a bacterium, protozoon parasite, or filterable virus. In contradistinction to louping-ill, "tick-borne fever" cannot be induced in mice, and we have so far been unable to transmit the infection to any of the ordinary laboratory animals. The infective agent is always present in the blood of affected sheep during the period of fever, and for prolonged periods after the reaction has subsided. By experimental inoculation its presence has also been demonstrated in the mesenteric glands, spleen and central nervous system of affected animals. The difficulty which we have experienced in demonstrating the infective agent suggests that it may be similar in its nature to the cause of tick-bite fever in South Africa, which, after prolonged study, has been shown by Pijper and Dau (1930) to be due to a living virus of the *Rickettsia* class. The following experiments were carried out in four groups of sheep, each of which is described separately.

Group I.

The Field Reaction in the Louping-Ill Immune Sheep.

The sheep in Group I were the animals used for the serial passage of the febrile affection which occurred in the louping-ill immune sheep while exposed to natural infection. For the purpose of determining the nature of this fever, blood was obtained from one reactor (*vide* Gordon *et al.*, 1932, sheep serial No. 56) on April 15th, 1931, when its temperature was 107·8° F. To facilitate description the blood thus obtained was named "immune reactor's blood" (I.R.B.). This blood was incapable of setting up louping-ill infection in sheep by intracerebral inoculation, and it was therefore assumed to be free from the louping-ill virus. A normal sheep was inoculated subcutaneously with 5·0 c.c. of this blood, and a temperature reaction commenced after an incubation of four days. Blood obtained from this animal during its reaction was inoculated subcutaneously into a second sheep which also reacted, and thereafter the infection

was passed in series through 26 animals. The temperature charts of five of these sheep are depicted in series in the left hand column of Chart 1, and the subsequent history of these sheep can be obtained by reading the charts from left to right. It will be observed that these five animals were tested with virulent " immune reactor's " blood at varying intervals after the primary reaction. As a control for the infectivity of each blood sample used for testing immunity, a normal sheep was also inoculated, and the fever curve exhibited by the control is depicted immediately above the chart of the tested sheep. For example, sheep No. 216 produced a typical primary fever curve after inoculation with I.R.B., and when tested with I.R.B. 30 days after recovery it was shown to have acquired a degree of immunity compared with its control depicted immediately above it. Twenty-nine days later it was again tested with I.R.B., and was practically immune. The record of the other four sheep shows that they were completely immune when first tested.

It may be concluded, therefore, that the reaction which occurred in the louping-ill immune sheep while exposed to natural infection was due to a specific infective agent which could be passed in series from sheep to sheep indefinitely. It may also be concluded that a specific immunity to this reaction can be produced in sheep.

Group II.

The Field Reaction of the Louping-Ill Non-Immune Sheep (" Controls ").

The Group II sheep were those used for the serial passage of the febrile affection which occurred in the louping-ill non-immune sheep while these were exposed to natural infection. As stated in a previous paper (Gordon *et al.*, 1932), it was believed that this febrile reaction was in some cases due to the louping-ill virus, and in others to this virus with, in addition, the infective agent responsible for the reaction in the louping-ill immune sheep. Blood, which we designated " control reactor's blood " (C.R.B.), was obtained from one of these reactors (*vide* Gordon *et al.*, 1932, sheep Serial No. 79) on April 15th, 1931, when its temperature was 107·4° F. This sample was proved to be free from louping-ill virus by intracerebral inoculation of sheep. By subcutaneous inoculation of 5·0 c.c. of this blood a reaction was produced in sheep, and passed in series through 27 animals. The charts of five of these are shown in series on the left of Chart 2. The subsequent history of each sheep can be obtained by reading the charts from left to right. It will be noted that all five animals were tested with virulent " control reactor's blood " at varying intervals after the primary reaction. It will also be observed that three were immune to the first test inoculation and the remaining two comparatively so, when compared with their controls.

It may be concluded from these experiments that, as in Group I, the infective agent responsible for the " reaction of controls " could be passed in series from sheep to sheep indefinitely, and immunity to this reaction could be produced.

Group III.

Animals used for Inoculation with Blood obtained from Sheep reacting to the Bite of Infective Ticks.

Blood of this nature was named " tick-borne fever blood " (T.B.F.B.), and was obtained from sheep reacting after infestation with ticks collected either from the pasture of the " diseased farm," or from infected sheep. Febrile reactions were regularly produced in normal sheep by subcutaneous inoculation with this type of blood, and these reactions were similar in nature to those produced in the sheep of Groups I and II. Five such reactions are depicted on the left of Chart 3, which also shows that in some cases sheep were immune after one inoculation, but in other cases more than one was required to produce immunity. Thus, as in the previous experiments, immunity can be demonstrated after recovery from the infection.

Group IV.

Sheep infested with Ticks collected either from the Sheep or from the Pasture of a Louping-Ill Farm.

The left hand column of temperature charts in Chart 4 are examples of the type of febrile reaction which was produced by infesting normal sheep with ticks collected from a louping-ill farm. This type of reaction was probably the one mistaken in some cases by Stockman (1918) for louping-ill, and shown by MacLeod (1932) to be immunologically distinct from that disease. Chart 4 also records the result of re-infesting some of these sheep with presumably infected ticks, and shows that they eventually became immune to tick bite, though several infestations were sometimes required. This is the febrile affection which we have named " tick-borne fever."

From these experiments it may, therefore, be concluded, as in the case of the reaction produced in Groups I, II and III, that a specific immunity to " tick-borne fever " caused by the bite of infective ticks can be produced in sheep.

Immunity Experiments between the Four Reactions.

In the foregoing experiments it has been shown that febrile reactions were produced in sheep with infective material obtained from four different sources. In the case of the reactions produced

in series in the animals of Groups I and II, the infective blood was obtained respectively from a louping-ill immune sheep and a louping-ill non-immune sheep which developed a febrile reaction while grazing on a louping-ill farm. The Group III reactions were produced with blood obtained from sheep which were reacting to the bite of ticks collected from the " diseased farm," and the Group IV reactions were " tick-borne fever," produced by infesting sheep with ticks collected from the same farm. The first two reactions were field reactions, the nature of which had to be determined, while the last two reactions were experimentally produced, and were believed to be " tick-borne fever." In order to determine that the two field reactions were the same, and that they were manifestations of " tick-borne fever " infection, immune sheep from each of the four groups were tested for immunity to the type of reaction produced in the three other groups.

Thus in Chart 1, No. 216, it is shown that a sheep which had produced a typical fever curve after inoculation with " immune reactor's blood " was comparatively immune when tested thirty days later, and almost completely immune when tested 29 days after the second reaction, while controls infected at the same time with the same material showed typical fever curves. The same sheep, when inoculated with " control reactor's blood " on two occasions, was completely immune. It was twice shown to be immune to " tick-borne fever blood," and when infested with known infective ticks was again immune. Chart 2, No. 343, shows that a sheep immune both to " control reactor's blood " and " immune reactor's blood " was immune to infection with known infective ticks. In Chart 3 there are several examples to show that sheep immune to " tick-borne fever blood " were immune both to " control reactor's blood " and to " immune reactor's blood," while Chart 4 shows that sheep which had recovered from the reaction caused by the bite of infective ticks were immune to inoculation with " tick-borne fever blood," as obtained from the sheep of Groups I, II and III.

As these experiments have been carried out a number of times, always with results as depicted in the Charts, it may be concluded that the field reactions which occurred in the louping-ill immune sheep while grazed on the " diseased farm " were due to " tick-borne fever " infection. In the case of the reaction in the louping-ill non-immune sheep it was probably due to this disease, with, in addition, louping-ill infection in some cases.

Immunological Difference between Louping-Ill and " Tick-Borne Fever."

Greig *et al.* (1931) and Gordon *et al.* (1932) showed that sheep which develop a febrile reaction after subcutaneous inoculation with louping-ill virus are immune on recovery to intracerebral infection

with this virus. Nos. 194 and 187, in Chart 1, and 185 in Chart 2 show primary curves after subcutaneous inoculation with louping-ill virus. After recovery these sheep were not immune to "tick-borne fever," and their final charts show that they were immune to intracerebral inoculation with louping-ill virus. Conversely, there are numerous examples throughout the four Charts which show that sheep immune to "tick-borne fever" were still susceptible to louping-ill infection. Since "tick-borne fever" can be induced in sheep immune to louping-ill, and louping-ill can be induced in sheep immune to "tick-borne fever," it may be stated that the two diseases are immunologically distinct.

Discussion.

MacLeod and Gordon (1932, b) have shown that the tick *Ixodes ricinus* L., is the vector of louping-ill virus. We now know that the same tick transmits the infective agent of "tick-borne fever," and in the course of other experiments we have encountered instances of both infective agents being harboured and transmitted by the same individuals. The close association of these two infective agents under natural circumstances may eventually prove to be of considerable importance in the etiology of louping-ill, especially in the production of those cases in which there is invasion of the central nervous system with virus. The only certain method of producing a typical clinical case of louping-ill by inoculation is by introducing the virus directly into the nervous system. If the virus is inoculated subcutaneously it is only in rare instances that it can pass the vasculo-meningeal barrier and be demonstrated in the central nervous system. The presence of the "tick-borne fever" infective agent in the brain of infected sheep suggests the interesting possibility that it may, in some way, favour the invasion of the central nervous system with the louping-ill virus. This aspect of the problem is at present under investigation.

Summary and Conclusions.

There occurs in the sheep on at least some of the tick-infested farms of Scotland a hitherto undescribed disease. When transmitted by inoculation it is characterised by an incubation period of about four days, followed by a febrile phase which lasts about ten days. The presence of the infective agent has been demonstrated in the blood, spleen and central nervous system. The mortality incidence is low, and after recovery most animals are comparatively immune to further infection. In animals killed during the febrile phase the only pathological change observed is splenic enlargement. The infective agent is transmitted by the tick *Ixodes ricinus* L. This condition we have, therefore, named "tick-borne fever."

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ABBREVIATIONS IN CHARTS.

I.R.B.	=	Louping-ill immune reactor's blood. That is, blood obtained from a louping-ill immune sheep which developed a febrile affection while grazing on a tick infested farm.
C.R.B.	=	Control reactor's blood. That is, blood obtained from a control sheep which had developed a febrile affection while grazing on a tick infested farm.
T.B.F.B.	=	Tick-borne fever blood. That is, blood obtained from a sheep reacting to tick bite.
Ticks	=	Infestation of sheep with ticks.
L.I.V.	=	Louping-ill virus.
+	=	Death.
---□---	=	The number in the squares between the temperature charts indicates the period in days which elapsed between the end of one reaction and the next inoculation.

CHART I.

CHART II.

CHART III.

CHART IV

INFESTED WITH NYMPHS FROM THE PASTURE
249.
245.
INFESTED WITH FEMALES FROM THE PASTURE.
278
250.
INFESTED WITH NYMPHS BRED FROM LARVAE FED ON INFECTED SHEEP.
286.
INFESTED WITH FEMALES BRED FROM NYMPHS FED ON INFECTED SHEEP.
288
323.
CONTROL.
T.B.F.B.
C.R.B.
I.R.B.
TICKS.
U.V.

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typical reaction after subcutaneous inoculation of sheep with louping-ill virus.

A FIELD EXPERIMENT - 1931

Phenomena which occurred in a group of Louping-ill Immune Sheep and a group of Susceptible Sheep when grazed on the Tick-infested Pasture of a Louping-ill Farm

In a field experiment ("Studies in Louping-ill.I) 24 sheep immune to louping-ill, together with a group of 25 susceptible sheep (controls), were grazed on a pasture where louping-ill is known to occur. During the period of exposure to infection (April 2nd till June 15th) the temperatures of all the sheep were recorded daily, and a daily count of the average number of fully gorged nymphal ticks on the head and ears of the sheep was made. The result of these observations is recorded in fig. III.

All the sheep became infested with ticks, and in every sheep, immune and control, a febrile reaction developed. The reaction in the immune sheep was not anticipated, and its cause was further investigated. A reprint of this work ("Tick-borne fever - A hitherto undescribed Disease of Sheep," by Gordon, Brownlee, Wilson & MacLeod) is attached, which provides evidence that there occurs in the sheep on at least some of the tick-infested farms of Scotland a hitherto undescribed disease. This disease is characterised by/

by a febrile reaction which lasts about ten days, during which period the infective agent is present in the blood. Besides being detected in the blood, the presence of the infective agent has also been demonstrated in the spleen and central nervous system. When transmitted by inoculation there is usually an incubation period of about four days prior to the febrile phase. The mortality incidence is low, and after recovery, most animals are comparatively immune to subsequent infection. In animals killed during the febrile phase the only pathological change observed was splenic enlargement. The disease was shown to be transmitted by the tick, Ixodes ricinus L., and was therefore named "tick-borne fever."

The cause of the reaction in the control sheep was attributed in a large proportion of cases to the infective agent of tick-borne fever, together with the virus of louping-ill. The most important evidence in support of this contention was the fact that all the surviving controls had acquired immunity to tick-borne fever, whilst 60.9 per cent. were immune to louping-ill.

The following is an analysis of the mortality incidence which occurred in the two groups of sheep while they were exposed to natural infection:-

9 healthy sheep immune to louping-ill:	mortality	nil =	0	per cent.
15 convalescent immune sheep:	mortality	5 =	33.3	per cent.
24 immunised sheep:	mortality	5 =	20.8	per cent.
25 control sheep:	mortality	13 =	52.0	per cent.

The mortality incidence in the 24 immunised sheep was confined to fifteen of them which were in a debilitated convalescent state following immunisation with living virus, and that some of them died when transferred to the experimental farm was not unexpected. The remaining nine immunised animals had completely recovered after immunisation, and although each developed a febrile reaction indicative of tick-borne fever infection, none of these sheep died. The tissues from all the control sheep which died were not examined because most of them showed no clinical evidence indicating central nervous system involvement, which was believed at that time to be the only manifestation of louping-ill infection. There is now evidence, however, that the virus of louping-ill may cause the death of sheep which present no symptoms indicative of invasion of central nervous system by the virus. Accordingly, the fact that many of the surviving control sheep had acquired immunity to louping-ill indicates that a natural infection with the virus had occurred during the period of exposure

to/

(3)

Studies in Louping-ill.

(AN ENCEPHALOMYELITIS OF SHEEP).

II.

TRANSMISSION BY THE SHEEP TICK,
IXODES RICINUS L.

BY

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Reprinted from
THE JOURNAL OF COMPARATIVE PATHOLOGY AND THERAPEUTICS.
Vol. XLV. Part 3. pp 240–256. September 1932.

Reprinted from
THE JOURNAL OF COMPARATIVE PATHOLOGY AND THERAPEUTICS.
Vol. XLV., Part 3, pp. 240—256. September, 1932.

STUDIES IN LOUPING-ILL

(An Encephalomyelitis of Sheep).

II.—TRANSMISSION BY THE SHEEP TICK, *Ixodes ricinus* L.

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The sheep tick, *Ixodes ricinus* L., has long been suspected of being concerned in the transmission of louping-ill, and during the last 50 years many attempts have been made by different investigators to incriminate it as the vector of the causal organism. In no case, however, was it definitely proved that louping-ill had been produced, and the evidence obtained was often of a conflicting nature. Within recent years considerable advances have been made in the study of the causation of louping-ill, and a knowledge of the new facts has made an investigation of the transmission of this disease a comparatively simple matter.

Until the cause of louping-ill was definitely established (Pool, Brownlee and Wilson, 1930, and Greig, *et al.*, 1931), the transmission experiments of earlier investigators were necessarily inconclusive, and their results difficult to interpret. The most significant results were those of Stockman (1918-19), who produced febrile reactions by infesting sheep with ticks obtained from affected farms. It now appears, however (MacLeod, 1932, and Gordon *et al.*, 1932), that ticks on louping-ill affected farms may harbour the causal agent of a hitherto unrecognised disease of sheep, "tick-borne fever." It is probable that the reactions produced by Stockman were in some cases due to tick-borne fever, and his results therefore cannot be considered to afford definite experimental proof of the transmission of louping-ill by ticks.

In a previous paper (Gordon *et al.*, 1932) the following conclusions were reached as a result of experimental investigations of the authors and others: (1) Louping-ill is due to a filterable virus which is communicable to mice as well as to sheep. In the mouse, characteristic effects and lesions are produced by it. Thus the use of the mouse constitutes a valuable biological test for detecting the presence of this

virus. (2) By this means it has been shown that blood drawn during the febrile phase of louping-ill in sheep contains the virus, but the virus may be present in an infected sheep without producing typical louping-ill. (3) Such atypical affections may be manifested by a febrile reaction only, or they may be a cause of sudden death in sheep. (4) Recovery from the infection, whether naturally or experimentally produced, results in immunity of the sheep to intracerebral inoculation with the virus. (5) The investigation of louping-ill is complicated by the fact that a distinct disease, tick-borne fever, may coexist with it. Tick-borne fever is clinically and immunologically distinguishable from louping-ill. (6) Blood from a sheep infected with tick-borne fever is capable of causing a specific temperature reaction in normal sheep. After recovery, there is immunity to inoculation with infective material. Mice are not susceptible to this infection.

The above data afford means of determining whether a temperature reaction, which is not followed by symptoms of nervous disturbance, is or is not due to louping-ill infection. Firstly, if the thermal reaction is due to louping-ill infection the blood of the reacting sheep is infective for mice; secondly, the sheep after recovery is immune to intracerebral inoculation of virus.

In the following paper evidence of the transmission of louping-ill by the tick, *Ixodes ricinus* L., will be given, showing that: (1) the virus of louping-ill was recovered from ticks which had fed on affected sheep; (2) the presence of virus was demonstrated in the tissues of nymphs which as larvæ had fed on affected sheep; (3) louping-ill was transmitted to sheep by the bite of female and nymphal ticks which had respectively acquired virus as nymphs and larvæ; (4) tick-borne fever probably aggravates the course of the louping-ill attack.

Transmission by Emulsion of Gorged Ticks.

Although the lesions of louping-ill appear to be confined to the central nervous system, the virus can be demonstrated in the blood stream for a definite period during the course of the disease (Pool *et al.*, 1930, and Gordon *et al.*, 1932). It is, therefore, probable that ticks will acquire virus when ingesting the blood of an infected sheep. If the presence of virus can be demonstrated in ticks which have fed on infected hosts, it then remains to prove whether virus thus acquired will survive through a moult, or through the egg stage.

During the course of a field experiment (Gordon *et al.*, 1932) ticks were collected from sheep exposed to natural infection with louping-ill and tick-borne fever, and these ticks furnished material for the following experiments.

Experiment 1.

A sheep under observation in the field developed louping-ill and virus was shown to be present in its tissues. Female and nymphal ticks, at various stages of engorgement, were removed from the sheep at death and stored

for 24 days, when they were washed in saline and emulsified in a sterile mortar. Sufficient saline was added to make a 1 in 10 suspension, and this was centrifuged for 15 minutes at about 2,000 r.p.m. The supernatant fluid was inoculated into mice intracerebrally, as indicated in Table I.

TABLE I.

RESULT OF INOCULATION OF MICE WITH AN EMULSION OF TICKS WHICH HAD FED ON A CASE OF LOUPING-ILL.

Number of Mice Inoculated Intracerebrally.	*Suspension of Emulsified Ticks in Saline Dilution.*	*Result.*
2	1 in 100	0 0
4	1 in 10	0, +11 T.L.I., ++13T.L.I.

2 Mice ++ 6 T.L.I.

3 Mice +++ 6 T.L.I.

0 = "No take."
+x = Death x days after inoculation.
T.L.I. = Typical louping-ill.

Experiment 2.

Ticks at various stages of engorgement were collected from a sheep which had developed a febrile reaction while exposed to natural infection. Following the febrile reaction this sheep died, and, although it had not shown symptoms of louping-ill, the ticks which had been collected from it were tested for the presence of louping-ill virus in the ingested blood. Fifteen days after collection some of the ticks were emulsified in saline and centrifuged, and the supernatant fluid was inoculated into mice, as described in Table II.

TABLE II.

A RECORD OF THE INFECTIONS RESULTING FROM THE INOCULATION OF EMULSIFIED TICKS. FOR PASSAGE OF VIRUS A SALINE SUSPENSION OF THE BRAIN OF THE INFECTED ANIMAL WAS USED.

Number of Mice Inoculated Intracerebrally.	*Suspension of Emulsified Ticks in Saline Dilution.*	*Result.*
2	1 in 10,000	0 0
2	1 in 1,000	0 0
2	1 in 100	0 0
2	1 in 10	+ 9 T.L.I. + 18 T.L.I.

3 Mice +++ 6 T.L.I. — 2 Mice ++ 5 T.L.I.

1 Sheep + 5 T.L.I. — 2 Mice ++ 6 T.L.I. — 2 Mice ++ 5 T.L.I. — 1 Sheep + 5T.L.I.

2 Mice 1 in 10	++6 T.L.I.	2 Mice 1 in 100	+8+9 T.L.I.
2 Mice 1 in 100	++6 T.L.I.	2 Mice 1 in 1,000	++9 T.L.I.
2 Mice 1 in 1,000	++6 T.L.I.	2 Mice 1 in 10,000	++14 T.L.I.
2 Mice 1 in 10,000	+6+7 T.L.I.	2 Mice 1 in 100,000	0 0

Abbreviations as in Table I.

Inference from Experiments 1 *and* 2.

The foregoing experiments show that when ticks are fed on sheep infected with louping-ill virus the virus may survive in the body of the tick for a period of at least 24 days. It now remained to decide whether virus thus acquired can survive through a moult of the tick and so be demonstrated in the succeeding stage, and also whether an infected adult tick transmits the virus to its progeny.

Emulsion of Larvæ from Females Exposed to Infection.

Experiment 3.

Of the ticks collected from the sheep referred to in the previous experiment and shown to have ingested virus, one fully gorged female was set aside. The larvæ bred from this female were emulsified in saline, centrifuged, and inoculated into three mice. One died of the operation ; two remained healthy for 21 days, when a negative result was accepted.

TRANSMISSION BY EMULSIONS OF TICKS AFTER MOULTING.

(*a*) *Emulsion of Nymphs Bred from Larvæ Exposed to Infection.*

Experiment 4.

Larvæ bred from females collected from healthy sheep, that is, " clean " larvæ, were infested on an experimental case of louping-ill (Ram 5) and fed during the course of the temperature reaction preceding its death from louping-ill. The fully gorged larvæ were collected and bred to the nymphal stage.

Some of these nymphs were emulsified in saline and centrifuged, and the supernatant fluid was inoculated into mice, as described in Table III.

TABLE III.

A RECORD OF THE INFECTIONS RESULTING FROM THE INOCULATION OF EMULSIFIED NYMPHAL TICKS WHICH HAD FED AS LARVÆ ON AN INFECTED SHEEP.

Number of Mice Inoculated Intracerebrally.	*Suspension of Nymphal Ticks in Saline Dilution.*	*Result.*
3	1 in 20	+6 T.L.I. +8 T.L.I. +7 T.L.I.

6 Mice { +5 T.L.I. +5 T.L.I. +6 T.L.I. +6 T.L.I. +6 T.L.I. +6 T.L.I.

2 Mice { +6 T.L.I. +6 T.L.I.

Abbreviations as in Table I.

(*b*) *Emulsion of Adults Bred from Nymphs Exposed to Infection.*

Experiment 5.

A sheep under close observation in the field died of louping-ill and virus was recovered from its tissues. During the course of the temperature reaction which preceded death three gorged nymphs were collected. These moulted to adult ticks, yielding two females and one male. An emulsion of these ticks was inoculated into two mice, neither of which developed louping-ill.

Inference from Experiments 4 *and* 5.

It appears that, in the case of the larvæ at least, louping-ill virus ingested by this stage can survive a moult, and can be demonstrated in the bodies of the resulting nymphs. In view of the small number of experiments it cannot be concluded from Nos. 3 and 5 that a negative result would always be obtained under these conditions.

It remained to prove whether ticks thus infected could transmit the virus to sheep by biting.

Transmission by Infestation with Ticks.

In the following experiments ticks were allowed to acquire infection by feeding on experimentally produced cases of louping-ill. These ticks were then bred to the next stage, and allowed to feed on normal sheep.

The technique employed was as follows: The scrotum of the ram was clipped and washed. The unfed ticks were placed in a cloth bag which was tied over the scrotum. The bag was removed after 24 hours, by which time the majority of the ticks had become attached. When the engorgement period was nearly over the bag was replaced and the gorged ticks removed from it daily.

Experiment 6, *Ram* 245.

This sheep was infested with nymphal ticks two months old, bred from larvæ gorged on Ram 5, an experimental case of louping-ill. The temperature of Ram 245 was recorded daily during the experiment. This animal did not exhibit any constitutional disturbance and its temperature remained normal for a period of fourteen days, when a negative result was accepted. This result was unexpected, as ticks from the same lot produced louping-ill when inoculated into mice (*vide* Experiment 4).

Experiment 7, *Ram* 285.

This ram was infested on August 22nd with female ticks bred from nymphs which had fed on Ram 7, an experimental case of louping-ill. Four females completed engorgement. The temperature of Ram 285 was recorded daily (Chart I). It will be seen that a temperature reaction commenced on the third day after infestation. The curve was diphasic in character and the reaction lasted for eleven days. On August 28th and September 3rd, blood drawn from this sheep was inoculated intracerebrally into mice. The mice remained healthy, but the result could not be taken as negative evidence of the presence of virus in the blood, since we now know that the most suitable dates for recovery of virus from the blood of this sheep would have been August 25th, 26th and 27th (Gordon *et al.*, 1932). In order to decide the cause of the temperature reaction, blood was again drawn from this sheep on September 4th, and inoculated subcutaneously into a normal sheep and a sheep immune to tick-borne fever. Neither of these sheep reacted. Seventeen days after its return to normal, Ram 285 was tested for immunity to tick-borne fever by the injection of virulent blood, and on the fourth day after it developed a temperature reaction which lasted for nine days. After recovery from this reaction the ram was tested for immunity to louping-ill by intracerebral inoculation of virus and did not react, whereas two control sheep inoculated at the same time developed typical louping-ill and were destroyed on the sixth day.

Accordingly, although the virus of louping-ill was not recovered from the blood of this sheep, the temperature reaction which followed infestation was probably due to louping-ill infection, since it was proved that the reaction was not due to infection with tick-borne fever, and since the sheep had acquired immunity to louping-ill.

In view of the above two results, the possibility had to be considered that the ticks used were infective, but were not capable of setting up a severe infection unless the resistance of the sheep was weakened in some way, such as by loss of bodily condition or by the presence of another disease. Under natural circumstances, tick-borne fever occurs along with louping-ill and is transmitted by the female and nymphal stages of the tick. Subsequent infestation experiments were therefore carried out in which tick-borne fever was induced in the sheep subsequent to, preceding, or simultaneously with, their exposure to biological infection with louping-ill, *i.e.*, to infection as a result of tick bite.

Infestation both with Louping-ill Infected Ticks and Tick-Borne Fever Infected Ticks.

(*a*) *Louping-ill followed by Tick-borne Fever.*

Experiment 8, *Ram* 245.

This ram was the animal recorded in Experiment 6. It failed to react after infestation with nymphs which as larvæ had ingested blood from a sheep harbouring louping-ill virus (*vide* Experiment 6). Fourteen days later it was infested with nymphs known to harbour the infective agent of tick-borne fever. A typical tick-borne fever reaction commenced on the fifth day and lasted over 15 days (Chart II). On the sixth day, when the temperature was 107·2° F., blood was drawn and inoculated into four mice without producing louping-ill. Blood was also inoculated into a normal sheep and a sheep immune to tick-borne fever. The normal sheep reacted and the immune sheep did not. Ram 245 was subsequently tested for immunity to louping-ill and died in six days after a typical attack of the disease. In this case, therefore, a tick-borne fever infection did not activate louping-ill virus which presumably had been introduced at the first infestation with ticks.

(*b*) *Tick-borne Fever followed by Louping-ill.*

Experiment 9, *Ram* 277.

Ram 277 was infested with females and larvæ bred from nymphs and females which had gorged on a case of tick-borne fever in the field, and typical tick-borne fever developed eight days later. On each of the first six days of the reaction blood from this ram was tested for the presence of louping-ill virus by inoculation into mice, and all the mice remained healthy.

On the ninth day of the reaction, when the temperature was 107·6° F., this sheep was infested with some of the nymphs infected with louping-ill as larvæ by feeding on Ram 5. (Some of the same group of nymphs were shown to be infected when emulsified and inoculated into mice (*vide* Experiment 4), while others had failed to produce a reaction when allowed to feed on Ram 245, Experiment 6). Ram 277 had a sustained high temperature for the four days following this second infestation (Chart III). On the fourth day, blood was drawn and inoculated into two mice, which developed

typical louping-ill on the fifth and seventh day respectively after inoculation. To confirm the diagnosis, virus was recovered from these mice and passed in series to other mice in which characteristic louping-ill developed.

On the eighth day after the second infestation the sheep was weak and lay most of the time. On the following day it was unable to stand ; breathing was laboured, but there were no characteristic nervous symptoms of louping-ill. In the evening the sheep died, and post-mortem examination revealed no gross lesions which might account for death, the organs being apparently normal. Saline emulsions prepared from the brain and spinal cord were inoculated into mice and the virus of louping-ill was recovered from both organs.

(*c*) *Simultaneous Louping-ill and Tick-borne Fever.*

Experiment 10, *Ram* 248

Ram 248 was infested with females and larvæ which in their previous stage had fed on a case of tick-borne fever in the field. At the same time it was infested with some of the Ram 5 strain of nymphs infected with louping-ill as larvæ. Chart IV depicts the temperature reaction following infestation. During the second febrile phase the sheep was dull and almost continuously lay apart from its fellows. On the fifteenth day after infestation the sheep was observed to be trembling violently ; it walked with difficulty, and occasionally showed twitching of the lips. The following day the animal was recumbent and exhibited generalised tremor of the superficial muscles. When raised to its feet it could stand only for a few seconds. On the sixteenth day the sheep was unable to rise, the hind quarters being paralysed, and at this stage it was killed by bleeding. Blood was drawn during the first febrile phase and inoculated into two mice, both of which developed louping-ill on the seventh day. After the death of the sheep louping-ill virus was detected in the brain and spinal cord by inoculation of saline emulsions of these tissues into mice. Towards the end of the second febrile phase blood was drawn and inoculated into one normal sheep and one immune to tick-borne fever. The normal sheep developed a tick-borne fever reaction, while the immune did not react. This was accepted as evidence that tick-borne fever infection was present in Sheep 248.

Experiment 11, *Ram* 283.

During the field experiment already referred to, a sheep exposed on pastures where louping-ill was prevalent developed a typical tick-borne fever reaction. At the end of the reaction the sheep developed symptoms of louping-ill and was killed. Presumably, then, towards the end of the thermal reaction the sheep was suffering both from tick-borne fever and louping-ill.

Gorged ticks collected from this sheep during the febrile phase were bred to the next stage and yielded numerous larvæ and eleven females. These were placed on Ram 283. Five females and numerous larvæ gorged. On the second day after infestation the temperature of this sheep rose to 106·4° F., and it was 107° F. on the following day. It then fell to 105·6° F., and remained low for another day, when it rose to 106·2° F. For the following six days its temperature was above 105·8° F., being usually over 106° F. It then subsided and subsequently remained normal. The sheep showed no clinical symptoms of louping-ill apart from a slight dullness and definite loss in bodily condition. During the course of the first temperature reaction blood was drawn and inoculated into two mice. These developed louping-ill on the sixth and seventh days respectively. During the second febrile phase blood was drawn and inoculated into one normal sheep and one immune

to tick-borne fever. The normal sheep developed a tick-borne fever reaction, while the immune sheep did not react, and this was accepted as evidence of tick-borne fever infection in Ram 283. After recovery this ram was inoculated subcutaneously with 5 c.c. of virulent blood from a case of tick-borne fever and did not react, whereas a normal sheep inoculated with a similar quantity of the same blood reacted severely. The ram was later tested for immunity to louping-ill by intracerebral inoculation of virus, and did not develop a reaction during the following eight days; but on the ninth day it died of pneumonia. Two controls inoculated with the same material developed typical louping-ill and were destroyed on the sixth day.

Summary.

In three infestation experiments in which tick-borne fever was induced prior to, or simultaneously with, exposure to louping-ill infection, louping-ill developed in the experimental sheep. One of the sheep developed characteristic symptoms of the disease and died; one died without having shown typical symptoms; and one developed a thermal reaction but recovered. All three sheep were proved to have been infected with louping-ill virus, because (1) the specific virus was recovered from the blood during the febrile reaction, (2) the specific virus was recovered from the central nervous system of the two sheep which died, and (3) the sheep which recovered had acquired immunity to this virus.

INFESTATION WITH TICKS OF DIFFERENT AGES.

The results of the above five experiments suggested that sheep infected with tick-borne fever were more susceptible to infection with louping-ill by tick bite than were normal sheep. As an alternative explanation of the failure of the ticks to produce a louping-ill reaction in a normal sheep (Experiment 6), it might be suggested that for some time after the tick has moulted it is not capable of causing infection, *i.e.*, that there is an incubation period during which the virus is either not infective or has not reached the salivary glands of the tick. In order to test these two hypotheses, a further experiment was carried out. The material for this experiment was prepared as follows:—

A ram (No. 289) was inoculated intracerebrally with louping-ill virus and infested with larvæ on the following day. These larvæ were the progeny of gorged females collected from apparently healthy sheep in the field. The larvæ completed engorgement towards the end of the temperature reaction preceding death of the sheep, and they were collected and bred to nymphs. Another ram (No. 290) was inoculated subcutaneously with louping-ill virus, and four days later was infested with larvæ of similar history to those put on Ram 289. Gorged larvæ were recovered towards the end of the temperature reaction in the host and were bred to nymphs.

Experiment 12. *Rams* 284, 428, 429, 427 *and* 247.

The nymphs bred from the larvæ gorged on Rams 289 and 290 were used in three lots, each lot being of different age after moulting,

viz., (1) twelve days old, (2) 15 to 30 days old, and (3) 40 to 50 days old. Five rams obtained from a non-louping-ill farm were used for the experiment. For seven days before the sheep were infested their temperatures were recorded daily and found to be normal. The twelve day old ticks were placed on one ram, which was then inoculated subcutaneously with 5·0 c.c. of virulent tick-borne fever blood. The 15 to 30 day old ticks were placed on two rams, one of which was also inoculated with virulent tick-borne fever blood. The 40 to 50 day old ticks were placed on two rams, and one of these was also inoculated with virulent tick-borne fever blood. From this experiment is was hoped to determine (*a*) if there was an incubation period after moulting before the tick became infective, and (*b*) if the presence of tick-borne fever infection precipitated or aggravated a louping-ill infection.

Ram 284. *Infested with Ticks under Twelve Days old, and Inoculated Simultaneously with Virulent Tick-borne Fever Blood.*

Four gorged nymphs were recovered. The temperature rose on the fourth day after infestation, and on the sixth day was 107·4° F. It dropped on the eighth day to 105·2° F., then rose the following day to 107° F. On this day the sheep was observed to be dull. During the following two days the temperature dropped from 106° F. to 105·4° F., when the sheep was definitely dull. It lay down when being penned in the morning. At 4 p.m. it was in a state of generalised muscular tremor; it could still maintain stance when assisted to its feet, but staggered to the right, and was inclined to collapse to that side. It was then destroyed by bleeding. Throughout the course of its temperature reaction blood was drawn daily and inoculated into mice. Virus was demonstrated in the blood stream on the fourth, fifth, sixth, seventh, eighth and ninth day after infestation. After the sheep was destroyed virus was detected in the central nervous system by inoculation of saline emulsions of this tissue into mice.

Ram 428. *Infested with Ticks* 15 *to* 30 *Days old, and Inoculated Simultaneously with Virulent Tick-borne Fever Blood.*

Only two nymphs attached. The temperature of this sheep rose to 105·4° F., on the fourth day after infestation, and was 108° F. on the seventh day. For the next six days it remained over 106° F. During this period the sheep showed evidence of discomfort; breathing was hurried and there was a definite loss in physical condition. The temperature declined during the fourteenth and fifteenth days to 104° F., rose to 105·6° F. on the sixteenth day and then dropped to 103·2° F. The following day the temperature was 102·4° F., and the sheep was unable to rise, breathing was rapid, and there was occasional violent kicking with the hind limbs. Intelligence was normal and the eye bright. It was destroyed by bleeding. Virus was recovered from the blood on the fourth day, and after death, from the brain and cord.

Ram 429. *Infested with Ticks* 15 *to* 30 *Days old.*

Seven gorged nymphs were recovered. This ram gave a prolonged and irregular temperature reaction from the fifth to the twenty-sixth day after infestation. The temperature did not exceed 106° F., except on the eighth day, when it was 106·2° F. Virus was present in the blood on the sixth

and seventh day after infestation. Forty-one days after infestation this sheep was tested for immunity by intracerebral inoculation of louping-ill virus and remained normal.

Ram 427. *Infested with Ticks* 40 *to* 50 *Days old, and Inoculated Simultaneously with Virulent Tick-borne Fever Blood.*

Nine gorged nymphs were recovered. This ram developed a thermal reaction on the third day after infestation and inoculation, and its temperature was 107° F. on the eighth day. The following day, the temperature dropped to 104·8° F. The sheep was excited, and when walking showed excessive flexion of the hind limbs. Occasional swaying was apparent in its gait. The following day the inco-ordination of gait was more exaggerated ; the animal trembled on being handled, and it was seized with a violent convulsion when its temperature was being taken. The symptoms persisted for the following two days, after which it made a gradual recovery. Virus was present in the blood on the third, fourth, fifth and sixth days after infestation. Forty-one days after infestation, the ram was inoculated intracerebrally with louping-ill virus and remained normal.

Ram 247. *Infested with Ticks* 40 *to* 50 *Days old.*

Sixteen gorged nymphs were recovered. Chart V shows the reaction of this sheep, which died as a result of infection with louping-ill alone. On the fourth day the animal was observed to be dull ; during the following two days it was distinctly dull and stumbled occasionally when walking. It was inclined to lie apart from its fellows and did not feed. There was a marked loss in condition. On the seventh day, when the second febrile phase began, a fine tremor of the limbs was apparent ; the ram was disinclined to move and stood with drooping ears and nodding head or lay apart. There was slight salivation. The following day the temperature dropped to 102·4° F. The animal was very dull and almost unable to stand ; it refused to be driven and when left alone lay down. Later it was unable to stand. Death occurred on this day. Virus was present in the blood on the third, fourth, fifth, sixth and seventh day. After death, virus was recovered from the spleen, but not from the brain or cord.

From these results it appears that nymphs infected with louping-ill as larvæ can produce the disease as soon after moulting as they are capable of attaching themselves to sheep, that is, there does not appear to be an incubation period after the moult during which the tick is incapable of causing infection. Further, it is clear that in the transmission of louping-ill tick-borne fever is not a necessary factor, although the latter disease, by reducing the sheep's vitality, may predispose it to develop the typical nervous symptoms of louping-ill.

Discussion.

The occurrence of the tick, *Ixodes ricinus*, on the rough hill pastures of Scotland and its association with louping-ill has long been recognised. We can find no authenticated record of the occurrence of louping-ill on farms which are free from ticks, while on badly infected louping-ill farms ticks are very prevalent. Further, it is known that the louping-ill season corresponds with the period of activity of the ticks in nature. Scientific proof, however, of the transmission of the disease by the tick had hitherto been lacking.

The investigation of the biological transmission of louping-ill has been complicated by the fact that under natural conditions ticks on "diseased" farms harbour and transmit two infective agents, the virus of louping-ill and the infective agent of tick-borne fever.

In the experiments described in the preceding pages it has been shown that when ticks engorge on a sheep affected with louping-ill during the period of thermal reaction virus can be recovered from these ticks. Further, the virus thus acquired by larvæ is present in the succeeding nymphal stage.

A number of experiments are recorded here in which ticks infected with the virus in one stage were allowed in their next stage to feed on healthy sheep. In these infestation experiments the following were taken as criteria of louping-ill infection :—

(*a*) The sheep develops a febrile reaction, which may or may not be followed by symptoms of nervous disturbance and by death.

(*b*) The blood of a reacting sheep, drawn at the acme of the thermal reaction and inoculated intracerebrally into mice, produces symptoms of louping-ill followed by death.

(*c*) A sheep which has recovered from a febrile reaction caused by louping-ill infection is subsequently immune to the virus inoculated intracerebrally.

The failure of the first attempt to transmit the disease to a healthy sheep by tick bite led us to look for a predisposing factor, and it was found that sheep affected with tick-borne fever readily became infected with louping-ill when bitten by infected ticks. Later experiments, however, showed that tick-borne fever is not a necessary factor, since healthy sheep may acquire louping-ill as a result of the bite of infected ticks.

Nine cases of louping-ill were produced in sheep by infestation with ticks infected with louping-ill virus.

In six of these nine, the sheep were infected with tick-borne fever, either before, or simultaneously with, exposure to louping-ill infection. Three of the six exhibited the classical symptoms of louping-ill and died or were destroyed when moribund ; one died after exhibiting atypical symptoms, one exhibited symptoms of nervous derangement but recovered, and one recovered after undergoing a thermal reaction only. Louping-ill virus was recovered from the blood of all six sheep ; it was present in the central nervous system of the four which died, and the two which survived were subsequently immune to the disease.

Three cases of louping-ill were produced in which tick-borne fever was not a concurrent infection. One of the three sheep exhibited atypical symptoms and died ; virus was recovered from its blood and spleen. Two recovered after developing a thermal reaction, and were subsequently immune to the disease. Virus was recovered from the blood of one of these during the course of the reaction.

It appears from these results that infection of the central nervous

system occurred only in those sheep which were undergoing a concurrent infection of tick-borne fever, and it is possible that the presence of this disease facilitates the invasion of the central nervous system by the louping-ill virus. It has been shown, however, that sheep can become infected with, and even die from, louping-ill infection by tick bite, although tick-borne fever be not present.

There is little doubt, in the light of these results, that the tick, *Ixodes ricinus* L., is the common vector of louping-ill in nature, and it is probable that, in at least the majority of infections, tick-borne fever plays an accessory part in establishing the disease.

The type of reaction in a sheep infected by tick bite appears to be as follows : There is an incubation period of usually one to two days, after which the temperature rises. The maximum temperature is reached about the second day of the reaction, and then the temperature declines during the following two or three days. Death may occur at this stage, or there may be a second rise and fall of temperature. This second febrile phase is accompanied by the appearance of the first symptoms of nervous derangement, if these develop. Recovery, or an increase in the severity of the symptoms and ultimate death, may follow.

Ticks were found to be capable of producing louping-ill within twelve days after moulting. Since ticks, after moulting, require about eight or nine days to harden before they will attach themselves to a host, it follows that they are infective as soon as they are able to attach themselves.

It is well established that there is considerable difficulty in producing louping-ill experimentally unless the infective material be introduced directly into the central nervous system. It is, therefore, a striking fact that the tick by inoculating the virus into the peripheral blood stream can cause infection of the central nervous system. This difference in the results of biological and artificial inoculation, and the possible rôle played by tick-borne fever in facilitating infection by the biological method of inoculation, raises interesting possibilities, and is at present the subject of further study.

The discovery that louping-ill can be transmitted by the tick is of interest in view of the fact that the causal agent of this disease is a virus which exhibits neurotropic characteristics. Until the work of Montgomery (1917), confirmed by Daubney and Hudson (1931), there was no record of ticks transmitting a virus disease. These workers reported the transmission of the virus of Nairobi Sheep Disease by a tick, *Rhipicephalus appendiculatus*, and that is the only other known instance of ticks transmitting an ultramicroscopic virus, but in its case the virus is not neurotropic.

Conclusions.

(1) Louping-ill has been transmitted to sheep by the bite of nymphal and adult female *Ixodes ricinus* ticks which in their previous stage had engorged on sheep affected with the disease.

(2) The tick is infective as soon after moulting as it is capable of attaching itself to a host.

(3) Evidence suggests that tick-borne fever may have an important influence on the course of the disease. In the absence of tick-borne fever the typical nervous symptoms of louping-ill are unlikely to develop, although infection with the virus of the latter occurs.

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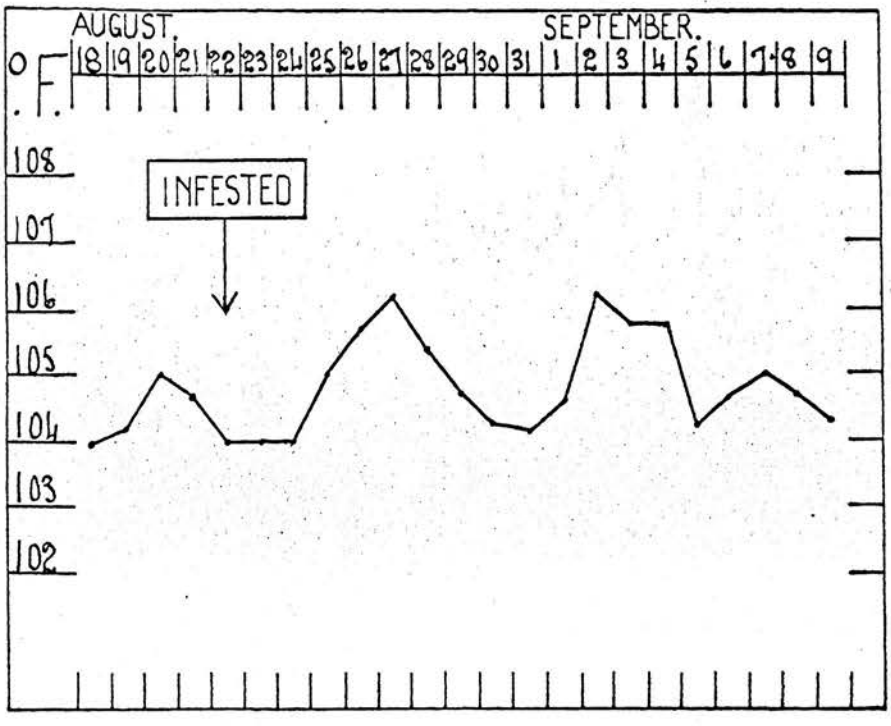
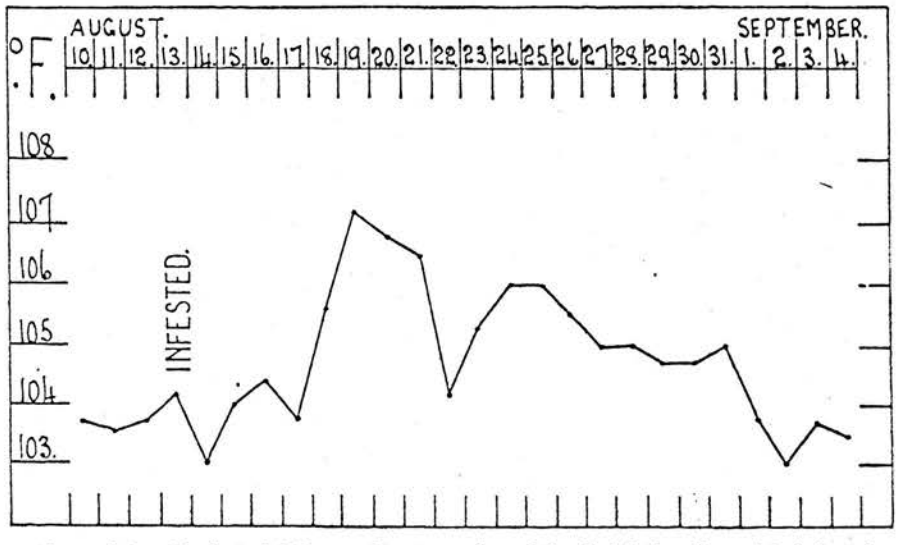
FIG 1.



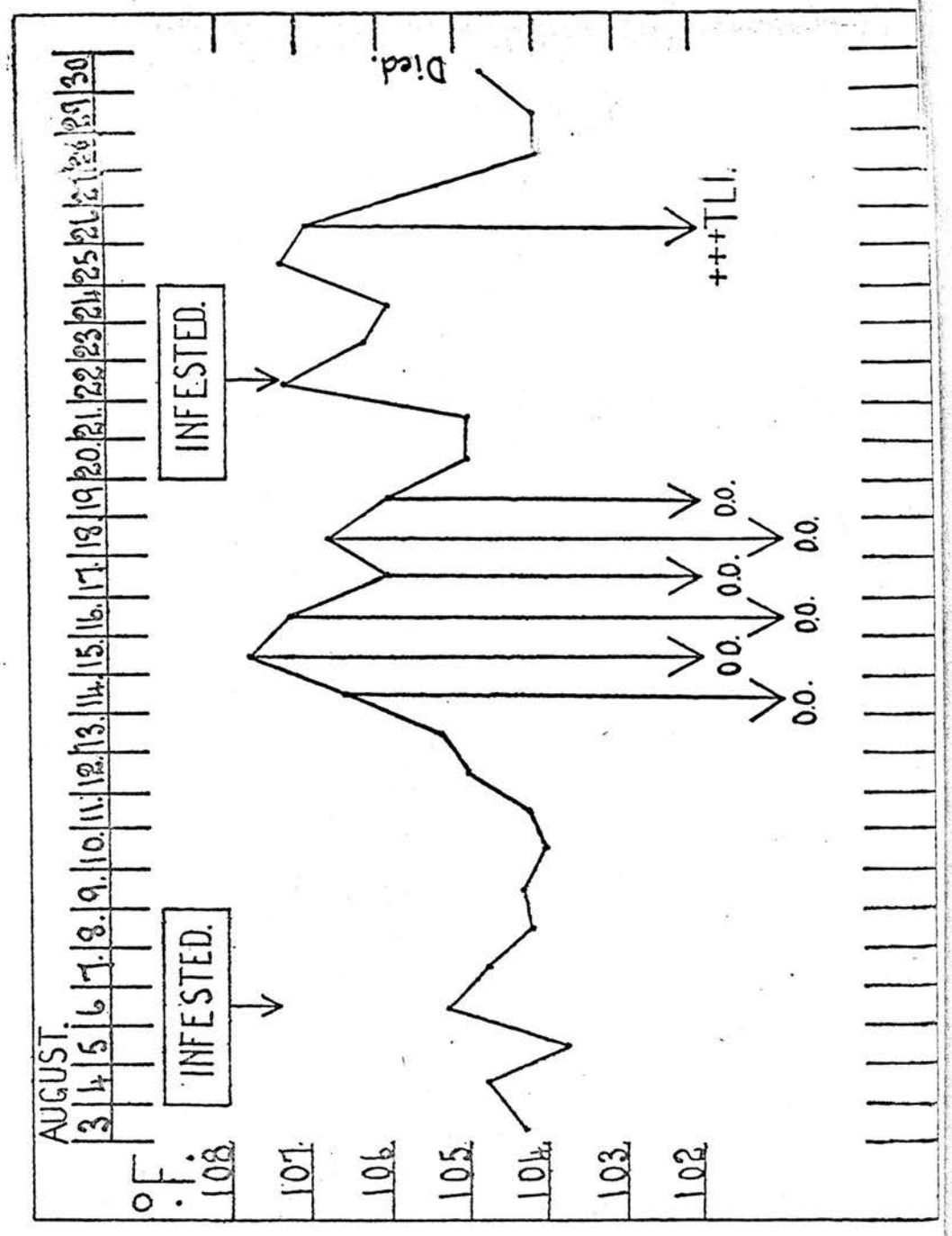
Sheep 285.—Temperature reaction following infestation with louping-ill infected ticks.

FIG 2.



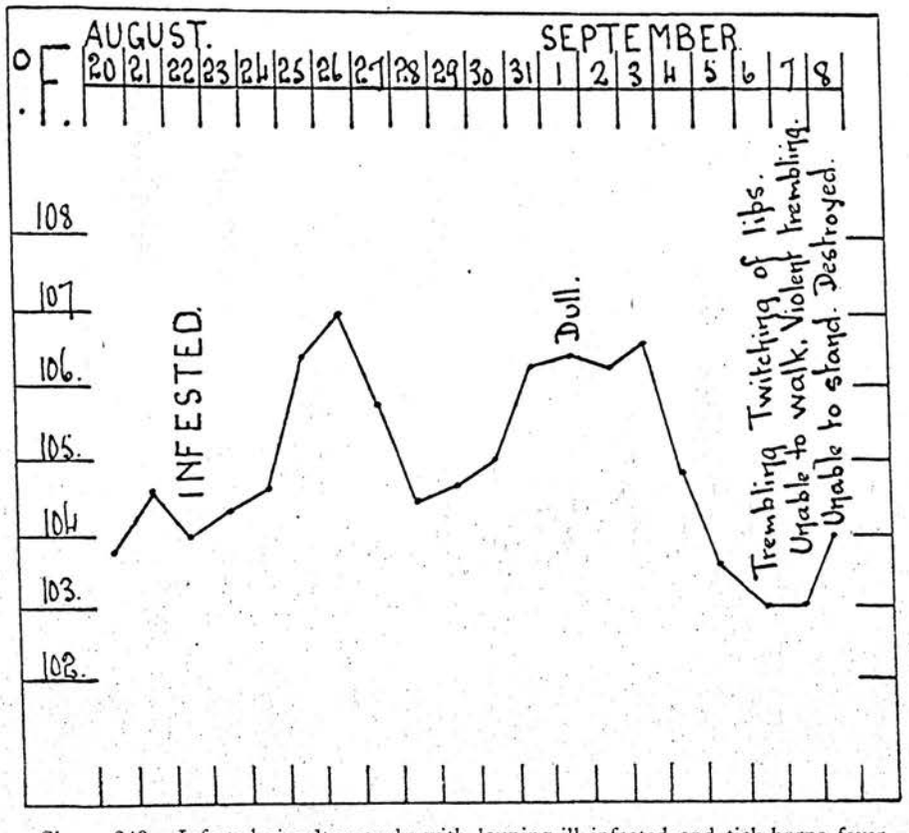
Sheep 245.—Typical tick-borne fever reaction, following infestation with infected nymphs.

FIG. 3.



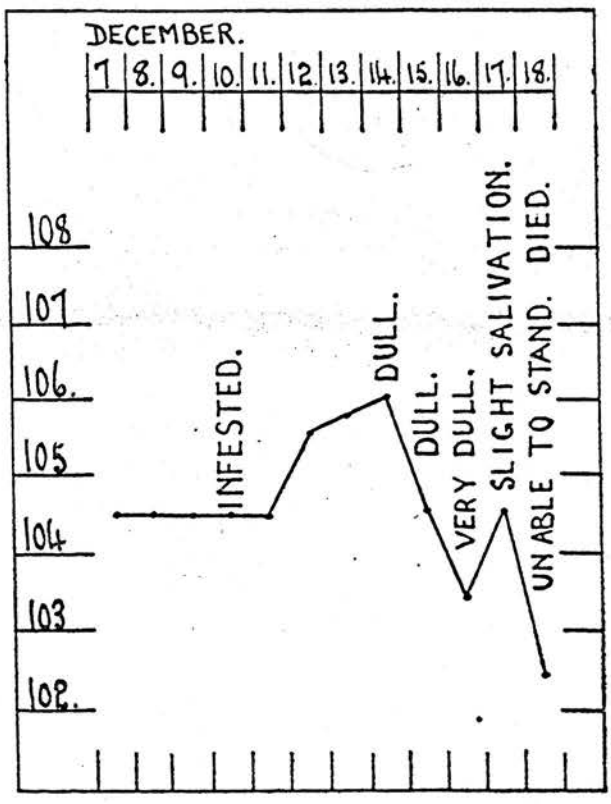
Sheep 277.—This sheep was infested with tick-borne fever infected ticks, and, during the subsequent reaction, was infected with louping-ill infected ticks. The arrows indicate the days on which blood was tested for the presence of louping-ill virus. " O " means " Mouse survived " ; " + T.L.I." means " Died of typical louping-ill."

FIG 4.



Sheep 248.—Infested simultaneously with louping-ill infected and tick-borne fever infected ticks. The sheep developed symptoms of louping-ill and was destroyed when moribund.

FIG 5



Sheep 247.—Temperature reaction and death as a result of infestation with louping-ill infected ticks, *i.e.*, without the presence of tick-borne fever.

H. R. GRUBB, LTD., PRINTERS, CROYDON.

to infection, and it is probable that many of the animals which died were primarily infected with the virus of louping-ill. These findings suggested that many sheep on a louping-ill farm acquire virus during the louping-ill season. Some develop a mild reaction, recover, and are subsequently immune. Some develop symptoms indicative of infection of the central nervous system, and it has been found that in these cases lesions of louping-ill can be demonstrated in, and virus recovered from, the brain and spinal cord. Experience, however, also indicates that in many cases no symptoms such as have been previously associated with louping-ill develop, and the sheep are found dead from undetermined cause.

During the course of the field experiment, ticks were collected from the experimental sheep. These ticks were subsequently bred to their next stage and tested for infectivity by feeding them on healthy sheep. All were found to be harbouring the infective agent of tick-borne fever, whilst a few were infected with louping-ill virus. These experiments are described in two papers by MacLeod and Gordon. The first paper (reprint attached: "Studies in Louping-ill." II.) details experiments which show that the tick, Ixodes ricinus, is the vector of louping-ill virus, and in the second paper (reprint attached: "Studies/

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STUDIES IN TICK-BORNE FEVER OF SHEEP

I. TRANSMISSION BY THE TICK, *IXODES RICINUS*, WITH A DESCRIPTION OF THE DISEASE PRODUCED

BY

J. MACLEOD AND W. S. GORDON

FROM PARASITOLOGY, VOL. XXV, No. 2, 14 APRIL, 1933

CAMBRIDGE
AT THE UNIVERSITY PRESS

PRINTED IN GREAT BRITAIN

[From PARASITOLOGY, Vol. XXV, No. 2, April, 1933.]

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By J. MACLEOD and W. S. GORDON.

From the Moredun Research Institute, Edinburgh.

(With 4 Temperature Charts.)

CONTENTS.

Introduction.

During the preliminary investigations into the transmission of a disease of sheep known as louping-ill, it was found that unfed nymphs of *Ixodes ricinus* collected from pastures in louping-ill districts were capable of causing an acute febrile reaction in sheep (MacLeod, 1932). This reaction was not considered to be due to louping-ill infection, as after recovery the animals were not immune to louping-ill. It was found also that a febrile reaction immunologically distinct from louping-ill occurred in sheep grazed on a tick-infested farm (Gordon *et al.* 1932 *a*). This febrile condition was further studied, and it has now been shown (Gordon *et al.* 1932 *b*) that the reaction was identical with that produced experimentally by MacLeod. The condition has been named tick-borne fever. When transmitted by inoculation of blood from affected sheep the disease is characterised by an incubation period of about 4 days, followed by a febrile phase which lasts about 10 days. The presence of the infective agent has been demonstrated in the blood, spleen and central nervous system of affected animals. The mortality is low, and after recovery most animals are relatively immune to further infection. In animals killed during the febrile phase the only gross pathological change observed was splenic enlargement.

In the present paper we describe: (1) experiments on the transmission of tick-borne fever by ticks collected from pasture, and by ticks which in their

previous stage had engorged on sheep affected with the disease; (2) experiments in the transmission of the disease by inoculation of infective ticks; (3) the duration of infectivity of the blood of recovered animals; (4) infection of the goat with tick-borne fever; (5) the salient features of the disease, as produced by experimental infestation.

Transmission of tick-borne fever.

The descriptions of the different infestation experiments have been condensed, the information being given in the following order: the stage and number of ticks used; the day on which the temperature reaction (T.R.) began, and the duration of the reaction; the highest temperature recorded. There then follows a note on any further experiments, such as inoculation of blood into a tick-borne fever immune and a control normal sheep, for the purpose of proving that the reaction was due to tick-borne fever, or inoculation of blood into mice in order to test for the presence of louping-ill virus. The latter was carried out as follows: Blood drawn from the reacting sheep was diluted 1 in 5 with citrate solution; mice were inoculated intracerebrally with 0·1 c.c. each of the mixture and kept under observation for the following 21 days. (None of them developed louping-ill, whereas it is known that mice similarly inoculated with blood from a louping-ill case during its febrile reaction develop louping-ill usually in about 6 days (Gordon *et al.* 1932 *a*).)

Transmission by ticks collected from pastures.

Unfed nymphal and female ticks were collected from louping-ill pastures in May. In the following August they were allowed to engorge on healthy blackface tups, the scrotal sac method being used (MacLeod and Gordon, 1932).

Tup 249. About 60 nymphs attached. T.R. on 5th day; lasted 9 days. Highest temperature 107·2° F. (Chart I). Blood tested during the first 6 days of reaction, and louping-ill virus found to be absent.

Tup 245. Sixty-seven nymphs attached. T.R. on 5th day; lasted 15 days. Highest temperature 107·2° F. Louping-ill virus absent from blood tested on 6th day (temperature at this time 107·2° F.). On recovery sheep inoculated with louping-ill virus and found to be non-immune.

Tup 276. Infested with "clean" nymphs from stock bred on a hedgehog, as a control. Heavy infestation. No T.R.

Tup 250. Infested with females. T.R. on 14th day; lasted 5 days. Highest temperature 107° F. Blood tested at peak of reaction (15th day) did not contain louping-ill virus. On recovery sheep inoculated with louping-ill virus and found to be non-immune.

Tup 278. Infested with females. T.R. on 10th day; lasted 6 days. Highest temperature 107° F.

Transmission by ticks which had fed in their previous stage on affected sheep.

Numerous ticks, gorged on various cases of tick-borne fever which occurred in the field[1], were collected throughout the course of the reactions.

[1] For the history of these cases see the account of the 1931 field experiment (Gordon *et al.* 1932 *a*).

This material was bred in the laboratory and furnished ticks of which the histories of the hosts for the previous stage were accurately known. With these a number of infestation experiments were carried out, the results of which are recorded below. The term "infected" is here used to designate ticks which fed on a sheep during the course of its reaction. The "infected" ticks had all moulted to the next stage by the end of June; the infestation experiments were commenced early in August. In the case of the first six infestations the primary object was to secure transmission of the disease; accordingly females and larvae bred respectively from "infected" nymphs and "infected" females were used together. These six experiments were completed in August.

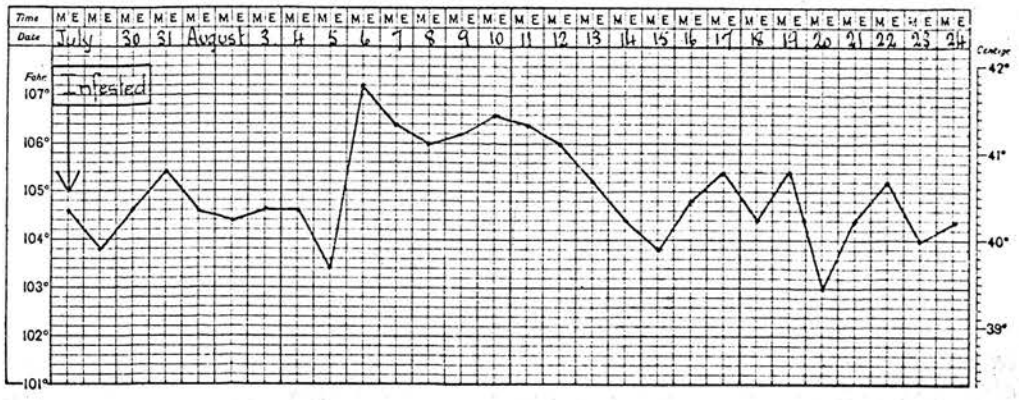

Chart I. Temperature reaction in Sheep 249 following infestation with nymphs collected from pastures.

Females and larvae.

Tup 275. (This lamb was in a debilitated condition.) Two females and numerous larvae attached. T.R. on the 10th day after infestation; highest temperature 105·8° F. The sheep died on the 16th day from bacillary necrosis of the liver.

Tup 277. Four females attached, and numerous larvae. T.R. on the 8th day; highest temperature 107·8° F. Blood was tested each day for the first 4 days of pyrexia and did not contain louping-ill virus. (Nine days after the beginning of the reaction the sheep was used for an experiment on the effect of tick-borne fever on louping-ill infection.)

Tup 246 (Chart II). Nine females attached, and numerous larvae. T.R. on 8th day; lasted for 13 days. Highest temperature 108° F. Blood from this sheep caused no reaction in a tick-borne fever immune sheep, No. 85, and caused a temperature reaction in a normal sheep, No. 293.

Tup 282. Two females and numerous larvae attached. T.R. on 10th day, lasting for 6 days. Highest temperature 107·3° F.

Tup 247. One female and numerous larvae attached. No T.R.

Tup 287. Two females and numerous larvae attached. No T.R. This sheep was subsequently shown by inoculation of infective blood to be susceptible to the disease.

Of the above six sheep infested with females and larvae bred from "infected" ticks, four reacted and two did not react. The explanation of the failure of these two to react is not known; one, at least, was susceptible, as

shown later by inoculatiou. It is to be remembered, however, that they were infested with only one and two infective ticks respectively; the larvae were non-infective, as shown later. With such small numbers of ticks the chances of the sheep becoming infected are reduced.

In the following experiments, which were carried out from the end of August onwards, the infectivity of each stage of the tick was tested separately.

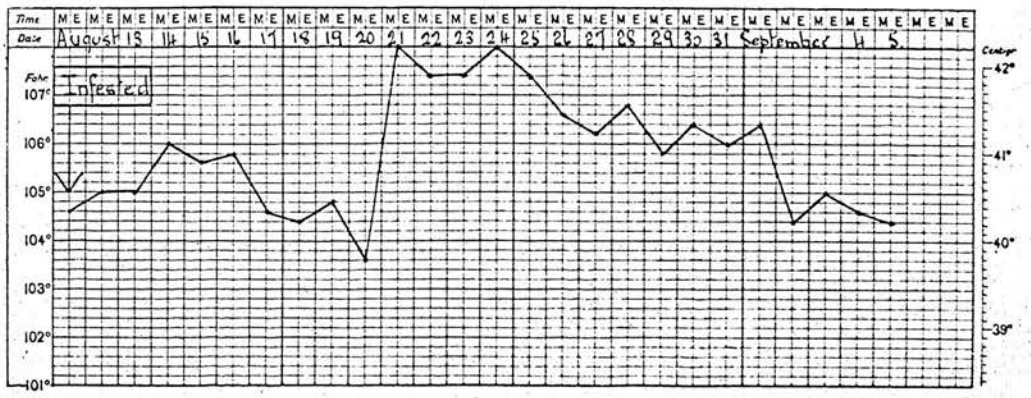

Chart II. Temperature reaction in Sheep 246 following infestation with females which as nymphs gorged on a case of tick-borne fever.

(*a*) *Transmission by females.*

Tup 288. Five females attached. T.R. on the 7th day; lasted for 12 days. Highest temperature, 107·4° F. Blood from this case caused in a normal sheep, No. 306, a T.R. which began on the 4th day and lasted 4 days.

Tup 323. T.R. on 7th day; lasted 11 days. Highest temperature 106·2° F. Louping-ill virus absent from blood tested at peak of reaction (8th day).

Tup 281. Fifteen females attached. T.R. on 8th day. Highest temperature, 107·8° F. Destroyed on 4th day of reaction. Louping-ill virus absent from blood and tissues.

Tup 276. Several females attached. T.R. 7th day; lasted 14 days. Highest temperature 106·8° F. Louping-ill virus absent from blood tested on 9th day. Blood inoculated into a normal sheep, No. 351, caused a T.R. on 5th day.

Tup 447. Several females attached. T.R. 7th day; lasted 22 days. Highest temperature 107·4° F.

Tup 244. Fourteen females attached. T.R. 6th day. Indefinite reaction; temperature did not exceed 105·2° F. Peaks on 6th, 16th and 23rd day.

Tup 500. Six females attached. T.R. 12th day; lasted 10 days. Highest temperature 107·4° F.

Tup 300. Several females attached. No T.R. This sheep was shown by subsequent inoculation with infective blood to possess a relative degree of immunity to the disease.

Tup 448. Several females attached. No T.R.

Tup 594. Five females attached. No T.R.

Of the above ten sheep infested with females bred from "infected" nymphs, six reacted definitely, one gave an indefinite reaction, and three did not react. One non-reactor was subsequently inoculated with infective blood and proved to be relatively immune. The other two were not tested further.

(*b*) *Transmission by nymphs.*

Tup 286. Thirteen nymphs attached. T.R. on 7th day; lasted for 12 days. Highest temperature 107·3° F. (Chart III). Louping-ill virus absent from blood tested on 8th day. Blood inoculated into a normal sheep, No. 308, caused a T.R. on the 4th day.

Tup 456. Six nymphs attached. T.R. 7th day. Highest temperature 107·6° F. Died on 6th day of reaction. No gross lesions which might have caused death. Louping-ill virus absent from blood. Blood inoculated into a normal sheep caused a typical tick-borne fever reaction. Brain and cord examined—no louping-ill lesions or virus present.

Tup 614. Eleven nymphs attached. T.R. on 7th day; lasted 14 days. Highest temperature 107° F.

Tup 615. Thirteen nymphs attached. T.R. on 8th day; lasted 14 days. Highest temperature 107° F.

Tup 244 (control experiment). Infested with nymphs bred from larvae gorged on hedgehog. No T.R.

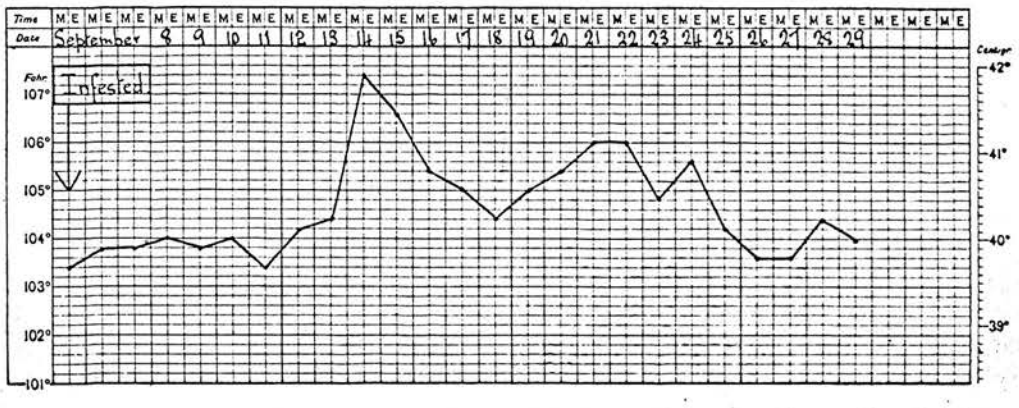

Chart III. Tick-borne fever reaction in Sheep 286 following infestation with nymphs infected as larvae.

(*c*) *Transmission by larvae.*

Eleven tups were infested, each with several hundred larvae bred from "infected" females, and none of them reacted. Eight of these were subsequently shown to be susceptible, four by infestation and four by inoculation; one was not tested further, and two were found to be immune when tested by infestation. In addition to this, there is the following negative evidence obtained before the disease was recognised:

Sheep 911, infested with larvae collected from pastures in an infected area, did not react.

Sheep 983 and 999, infested with larvae—the progeny of females engorged on sheep in an infected area—did not react.

It appears, therefore, that there is no hereditary transmission of tick-borne fever, *i.e.* the infection acquired by the female apparently does not pass through the egg stage of the tick.

Experiments on transmission by emulsions of ticks.

Exp. 1. Females and nymphs at all stages of engorgement were collected from two sheep reacting to tick-borne fever in the field. Fifteen days later these ticks were washed in saline, weighed and emulsified in a sterile mortar with sufficient sterile saline to yield a 1 in 10 dilution. This was centrifuged for 20 min. at about 2000 R.P.M., and the supernatant fluid, in varying dilutions, inoculated intracerebrally into mice. The mice remained healthy.

Exp. 2. A gorged female, collected from a case of tick-borne fever in the field, was allowed to oviposit in the laboratory, and the eggs hatched to larvae. 0·5 g. of these was ground up in sufficient saline to yield a 1 in 10 dilution. This was centrifuged as before and inoculated into mice, all of which remained healthy.

Exp. 3. Nymphs and larvae were allowed to feed on an experimental case of tick-borne fever (Tup 288). After engorgement, they were collected and kept in the laboratory for 24 days. They were then incubated at 30° C. for 24 hours, and the larvae and nymphs separately emulsified. Sufficient saline was added to each emulsion to yield a dilution of 1 in 10. These emulsions were centrifuged, and the supernatant fluid inoculated intracerebrally into mice. Of the mice inoculated with the nymphal emulsion, one died from the operation, one died in 8 days, and two survived. Of the mice inoculated with the larval emulsion, three survived and one died in 8 days. The deaths which occurred are believed to have been due to bacterial contamination.

It has already been shown that infective blood does not produce a reaction when inoculated into mice, thus the failure of the tick emulsions to produce a reaction in mice was to be expected. These results afford further evidence in support of the view that this disease is distinct from louping-ill, for it is known that mice react to intracerebral inoculation of emulsions of ticks infected with the louping-ill virus (MacLeod and Gordon, 1932).

In the following experiments the tick emulsions were inoculated subcutaneously into sheep. The unfed ticks were incubated before being emulsified, since it has been shown in the case of Rocky Mountain Spotted Fever that ticks which readily infect by biting are not regularly infective when emulsified and injected, unless previously incubated (Spencer and Parker, cited by Theiler, 1928).

Exp. 4. Seventeen unfed adults and some 2000 larvae, bred from nymphs and adults gorged on a field case of tick-borne fever, were incubated at 30° C. for 24 hours. They were then emulsified in saline with sterile sand and the emulsion, after removal of the débris by light centrifugalisation, was inoculated subcutaneously into a normal sheep, No. 310. This sheep did not exhibit a thermal reaction, and when subsequently tested with virulent blood was found to have developed no immunity to the disease.

Exp. 5. Gorged nymphs were collected from a number of field cases of tick-borne fever and bred to adults. Some of the females were allowed to feed on a normal sheep, No. 244, to test their infectivity. This sheep developed an indefinite attack of tick-borne fever. The remainder of the unfed ticks, consisting of seventy-three adults, were incubated for 24 hours at 25° C., weighed, and emulsified with sterile sand in enough saline to yield a 1 in 25 dilution. After sedimentation of the sand and heavier particles, the emulsion, amounting to about 4 c.c., was inoculated subcutaneously into a sheep, No. 407. This sheep did not react.

In view of the above negative results, the effect of allowing the tick to become partially engorged on a susceptible animal before inoculation was

tried. It has been shown by Theiler (1928) that preliminary partial engorgement of the tick is necessary in the case of East Coast Fever, and increases the probability of infection in the case of heartwater.

Exp. 6. Of the females which were allowed to feed on Tup 244 seven were removed when partially engorged and emulsified in saline with sterile sand. After sedimentation, the emulsion was inoculated into a normal sheep, No. 415. This sheep did not react.

Exp. 7. Females bred from nymphs gorged on a field case of tick-borne fever were allowed to gorge on a sheep, No. 447, and produced a typical tick-borne fever reaction. Eight days after infestation, when practically fully gorged, these ticks were removed from Sheep 447, and 0·25 g. was emulsified with sterile saline. The emulsion, after sedimentation of the heavier particles, was inoculated subcutaneously into a sheep, No. 481. The sheep did not react.

The failure of the tick emulsions to cause a reaction when inoculated subcutaneously into sheep is of considerable interest. Ticks which were known to be infective by biting failed to cause a reaction, either when emulsified as unfed ticks after incubation, or when emulsified after partial engorgement. That is, in the limited number of experiments carried out, the infective agent in the tick was not rendered virulent either by incubation at 25 or 30° C. or by the ingestion of blood by the tick.

Infectivity of blood of recovered cases.

It has previously been shown (MacLeod, 1932) that the blood of a recovered case was infective to sheep for a period of 44 days after subsidence of the thermal reaction. In order to test the duration of infectivity, the following experiment was carried out. Sheep 276 was infested with tick-borne fever-infected ticks, and reacted 7 days later, the reaction lasting 14 days. During the fever, blood was drawn from this animal and inoculated into a normal sheep which gave a definite reaction. Blood was drawn from No. 276 at different intervals after recovery, and tested in normal sheep, with the following results.

Fifteen days after recovery 5 c.c. of blood were inoculated into a normal sheep which developed a typical reaction. Thirty-five days after recovery, blood produced a slight temperature reaction in a normal sheep, the reaction lasting for only 4 days. Seventy days after recovery, the blood was found to be non-infective. It appears, therefore, that in this particular sheep, the blood remained infective for a period of between 35 and 70 days after recovery.

A sheep, No. 135, exposed in the field, developed a tick-borne fever reaction and recovered. Nymphs which had gorged on this sheep during the fifth week after subsidence of its reaction were bred in the laboratory, and the resulting females were allowed to infest Tup 292; this tup developed on the 8th day a temperature reaction which lasted for 8 days. The highest temperature recorded was 107·2° F. Louping-ill virus was absent from the blood when this was tested in mice on the 11th day.

It appears, therefore, that sheep which have recovered from an attack of tick-borne fever are reservoirs of infection for some considerable time, and

can infect the ticks which feed on them after their reactions are over. The alternative explanation of the reaction in Tup 292 is that the ticks acquired infection as larvae, and that the infection survived in the ticks through the nymphal stage which fed on Sheep 135 in the field. The possibility of infection lying latent through one stage of the tick has yet to be tested.

Tick-borne fever in the goat.

A goat, No. 49, was infested on the scrotum with nymphs bred from larvae which had fed on a field case of tick-borne fever. The normal temperature of the goat varies between 102 and 103° F. On the 10th day after infestation, the temperature of this goat rose to 106·6° F. The reaction lasted for 7 days, the temperature steadily subsiding during this time. By the 8th day, it was again normal (Chart IV).

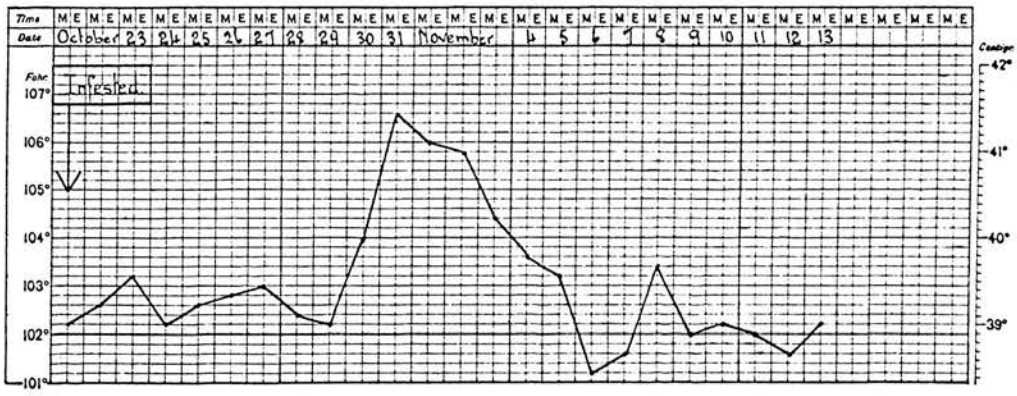

Chart IV. Tick-borne fever reaction in Goat 49 following infestation with infected nymphs.

Another goat, No. 206, was inoculated subcutaneously with 5 c.c. infective blood. On the 6th day, the temperature rose to 106° F. The reaction lasted for 6 days.

Goat No. 210, inoculated subcutaneously with 5 c.c. of infective blood, showed on the 5th day an indefinite reaction which lasted for 4 days. The temperature in this case did not exceed 103·6° F.

The goat, therefore, appears to be susceptible to tick-borne fever.

Description of the disease.

From the data given in the preceding text we may outline the chief characteristics of the disease, as it occurs after tick infestation, as follows:

Incubation period. The incubation period after infestation by infected nymphs appears to be shorter than that after infestation by females. In the former case it is usually 4–6 days but varies from 3 to 7 days. (In three cases produced by nymphal infestation, described in a previous paper (MacLeod, 1932), the periods were 3, 4 and 4 days respectively.) After infestation by

females, on the other hand, the incubation period is usually about 7–8 days, although it may vary from 5 to 13 days.

Febrile phase. After the incubation period there is an abrupt rise in temperature to between 106 and 108° F., the highest point of the thermal reaction being usually reached on the second day. The reaction is maintained for a period of from 6 to 22 days. The usual period of reaction, in the case of infection by infestation, is about 12 days. During the later stage of the reaction, the temperature gradually subsides to normal. A slight but appreciable fall preceding the initial rise in temperature is a frequent but inconstant feature of the reaction (see Charts I and II). In the case of infection by inoculation of infective blood the incubation period is shorter—usually 3–4 days—and the reaction is usually of shorter duration, the temperature falling more abruptly to normal.

Mortality. If uncomplicated by other infections, the disease seldom terminates fatally. Out of seventy-five cases produced by inoculation of infective blood, there occurred only two deaths directly attributable to tick-borne fever infection. Of the twenty experimentally produced cases of tick-borne fever in sheep recorded in this paper, two died (Tups 456 and 275). The autopsy of Tup 456 revealed no gross lesions which could be regarded as responsible for death. This sheep died apparently as a direct result of tick-borne fever infection. In the other case (Sheep No. 275), the immediate cause of death was bacillary necrosis of the liver.

Duration of infectivity of blood. The blood of a recovered case of tick-borne fever remains infective for sheep for some time after the reaction has subsided. In the case of one sheep, the blood was infective 35 days after recovery, but non-infective after 70 days. As recorded in a previous paper, blood was found to be infective when drawn 44 days after subsidence of the reaction. There is also evidence to suggest that ticks which feed on a recovered case acquire infection, and are infective in their next stage. Apparently, then, sheep which have reacted act as reservoirs of infection for some time after recovery.

Discussion.

In the course of an investigation into the transmission of louping-ill, it became apparent that the tick, *Ixodes ricinus*, was capable of causing a febrile condition in sheep. This condition was found to be due to an infective agent which could be passed from sheep to sheep in series. The disease so produced was shown to be clinically and immunologically distinct from louping-ill, and was named tick-borne fever. This paper describes the regular transmission of the disease by *Ixodes ricinus*. We have already shown that the same tick is the vector of louping-ill (MacLeod and Gordon, 1932); there is thus evidence that this tick may transmit the infective agents of two distinct diseases of sheep.

Although the mortality directly resulting from tick-borne fever is relatively low, the disease is nevertheless of considerable economic importance.

It is well known that in certain districts many sheep fail to thrive in the spring, even after there is a good growth of grass. The only clinical symptoms of illness in such sheep are dullness and loss of bodily weight. Lambs especially are said to pass through a period of unthriftiness after they are about 3 weeks old. Since this type of case occurs during the louping-ill season and is associated with the presence of ticks on the sheep, the farmer naturally attributes it to a mild infection with louping-ill. It is probable that many of these cases of so-called mild louping-ill are actually cases of tick-borne fever.

During this period on tick-infested farms it is common for a number of the sheep to die from undetermined causes, and it is possible that such deaths result from a primary infection with tick-borne fever followed by some secondary infection. Our experimental evidence (MacLeod and Gordon, 1932) shows that tick-borne fever aggravates the harmful effects of louping-ill infection, and it is probable that it may predispose sheep to other secondary infections, especially since it has a debilitating effect on the affected animal. It is therefore possible that the spring mortality which occurs among the flocks on at least some tick-infested farms may be due in many instances to secondary infections, the common predisposing cause being a primary tick-borne fever infection.

Summary.

1. The causal agent of tick-borne fever is transmitted by the adult female and the nymphal stage of the tick *Ixodes ricinus*. Two female ticks are sufficient to produce infection in a sheep. The larval stage does not appear to be infective. In a limited number of experiments the disease has not been produced in sheep by the inoculation of emulsions of presumably infective ticks.

2. The disease as produced by tick infestation is characterised by a period of incubation which varies from 3 to 13 days, after which there is a sharp rise in temperature; a "plateau" type of febrile reaction follows which lasts from 6 to 22 days, the temperature gradually subsiding to normal. Affected animals usually recover. Mortality as a direct result of infection is rare, but there is evidence to suggest that tick-borne fever may predispose affected animals to death from secondary causes.

3. The blood of a sheep which has reacted to the disease remains infective for some time after the subsidence of the thermal reaction.

4. The goat is susceptible to tick-borne fever.

5. The economic importance of tick-borne fever is discussed.

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(*MS. received for publication* 17. I. 1933.—Ed.)

"Studies in Tick-borne Fever. I.") the same tick is incriminated as the vector of tick-borne fever.

THE METHOD WHEREBY TICKS ACQUIRE INFECTION

At first it would seem a curious anomaly that a blood-sucking arachnid is responsible for the transmission of a neurotropic virus, but the apparent anomaly is explained by the fact that following infection with louping-ill, even when the specific virus is introduced intracerebrally, multiplication of the infective agent occurs in the blood at an early stage of the infection. This explains the mechanism whereby ticks may acquire the so-called neurotropic virus. Fig. IV is taken from a previous paper by the author (reprint attached: "The Control of Certain Diseases of Sheep") and shows diagrammatically the method adopted for the experimental infection of ticks with the virus of louping-ill.

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The Control of Certain Diseases of Sheep

BY

W. S. GORDON

From the Animal Diseases Research Association, Moredun Institute, Gilmerton, Edinburgh.

Reprinted from "THE VETERINARY RECORD" Vol. XIV. (New Series) pp. 1-8. January, 1934.

THE CONTROL OF CERTAIN DISEASES OF SHEEP*

W. S. GORDON, M.R.C.V.S.

ANIMAL DISEASES RESEARCH ASSOCIATION

There are a few facts concerning the control of certain diseases of sheep, and when one assembles these facts one realises what the veterinary profession has accomplished in the investigation of sheep diseases within the comparatively short period of twelve years. It has been said of research into human diseases that rarely in the lifetime of a research worker is he fortunate enough to discover the cause of a disease, and to determine methods for its prevention. It cannot be said that the same applies to the research worker in veterinary science, since, in the investigation of sheep diseases alone, the cause and prevention of two of these diseases in this country have been established within recent years. I refer to the work of Gaiger[1, 2] in establishing methods for the control of braxy in sheep, and to the work of Dalling[3, 4] in determining the cause of lamb dysentery, and in evolving the present-day methods for its control. Each of these diseases represented a major scourge to the sheep industry of Scotland, whereas now neither disease need be feared by the flockmaster.

As a profession, we owe a deep debt of gratitude to Gaiger and Dalling for their pioneer work in the investigation of sheep diseases in this country. They have enhanced the reputation of veterinary research, because they have given us concrete modern examples of the practical value of pathology and bacteriology in their application to the control of infectious disease. It is no exaggeration to say that Gaiger's work has saved hundreds, if not thousands, of sheep, and Dalling's discovery has saved thousands of lambs and rescued many sheep farmers from crippling losses. Their results strengthen the confidence of public opinion in the value of veterinary research, and so increase confidence in our whole profession. Their work also shows that we can accomplish a quicker application of biological products for

* Paper presented to the Scottish Metropolitan Division, N.V.M.A., at Edinburgh, October 25th, 1933.

the control of infectious diseases in animals than our fellow workers in medical science when applying their products to the control of disease in man. We are not faced with a long and difficult period of years in establishing the value of prophylactic vaccines; we can immediately apply the experimental products under carefully controlled conditions, and, if necessary, we can vaccinate one group of animals, leave a similar group untreated, expose both groups to the same risk of natural infection and observe the results. After a few years' experimentation on these lines, massive statistical evidence in regard to the efficacy of prophylactic vaccines can usually be obtained. This is one of the most valuable weapons we possess in determining our efficiency in controlling animal disease, and one which we would all benefit from making use of as often as the opportunity presents itself. In so doing we would prevent ourselves from developing any exaggerated notion as to our capabilities in preventing animal disease. In this connection the remarkable progress which has been made in the control and diagnosis of infectious disease with the aid of biological products, and the extensive use of the preparations, necessitate certain definite standards being adopted for the evaluation of their potency and purity.

In this country there is no Governmental supervision over the production of biological products; consequently the practitioner has no guarantee that the products he receives have been properly standardised: he merely trusts that they will perform the function expected of them. In America such blind faith no longer exists, since the enactment of the Virus Serum Toxin Law, and the production of veterinary biological products is now under state control. The report by Mohler[5] on "Standardisation of Veterinary Biologics in the United States" is a revelation of the chaos that can exist in a country where the producers have a free hand in issuing biological products without state supervision. Tetanus antitoxin for veterinary use was in some instances about two-thirds less than the strength the product should have possessed. Samples of imported hog cholera vaccine, and samples of hog cholera and swine

plague serum were found to be quite unreliable. A serum widely advertised for the prevention and cure of abortion in cattle was found to be a weak solution of carbolic acid, and not a serum at all. Diphtheria antitoxin, which had been returned to the producer because its date of usefulness had expired, was relabelled and issued as antitoxin for equine influenza, chicken roup, dog distemper, etc. Normal serum derived from animals slaughtered for food purposes was sold as anti-hog cholera serum, lymph for small-pox vaccination was found to be contaminated with the virus of foot-and-mouth disease, and so on. You might think that such a state of affairs would never occur in this country, but there is nothing to prevent it, and later in this discussion I will instance a case in which a serum, issued by a presumably reliable firm, and labelled "Lamb Dysentery Antiserum," had no more value in preventing the disease than had normal serum. In other words, the serum was quite non-specific and valueless.

The Control of Braxy

Gaiger[1, 2] showed that the mortality from braxy could be reduced by the immunisation of sheep against infection with *Cl. septique*, and in the Annual Report of the Animal Diseases Research Association (1923)[6] it is stated that of 10,340 hoggs inoculated, the mortality from braxy was 2·39 per cent., whereas out of 3,800 control hoggs the mortality from braxy was 9 per cent. The vaccine employed was a sterile filtrate of the growth of *Cl. septique* in liquid medium. This filtrate was toxic for sheep, but was rendered reasonably safe for use by dilution with carbol saline. At that time definite methods of standardisation for braxy vaccine had not been laid down; consequently it was difficult for anyone other than the originator of the method to produce exactly the same results.

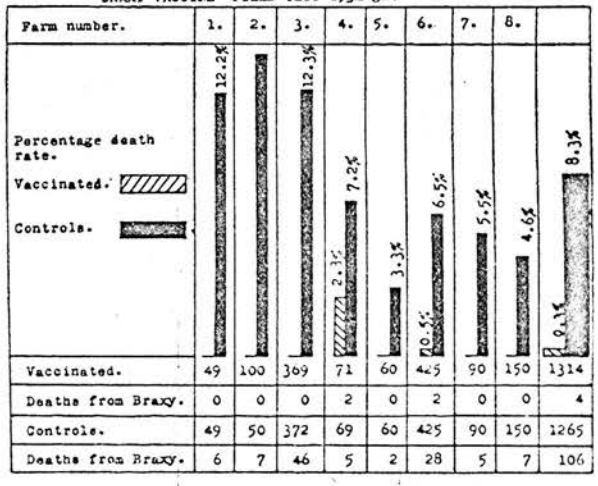
From 1923 onwards, research into the methods of production and standardisation of braxy vaccine have been in progress both at the Wellcome Research Laboratories and at the Moredun Institute. In addition to this work, cultural examination of hoggs on the point of death from a braxy-like disease has been made from time to time, and, as far as applies to

braxy in Scotland, the only anaerobic organism isolated in upwards of twelve cases in widely separated districts was *Cl. septique*. This suggests that if the vaccine employed in the control of the disease produces a definite immunity to *Cl. septique*, the incidence of braxy should be very much reduced. In the braxy season of 1930-31, we carried out experiments in which the comparative value of a filtrate vaccine and a formalinised whole culture vaccine was estimated. This experiment showed that the formalinised whole culture vaccine was superior to the filtrate vaccine, and so in the season 1931-32 we issued this type of vaccine for field use, and carried out a carefully controlled experiment on eight farms. The result of this experiment is shown in Fig. I.

In the season 1932-33 we issued 34,885 doses

FIG. 1.

BRAXY VACCINE FIELD TEST 1931-32.



Farm number.	1.	2.	3.	4.	5.	6.	7.	8.	
Vaccinated.	49	100	369	71	60	425	90	150	1314
Deaths from Braxy.	0	0	0	2	0	2	0	0	4
Controls.	49	50	372	69	60	425	90	150	1265
Deaths from Braxy.	6	7	46	5	2	28	5	7	106

of formalinised whole culture for the prevention of braxy, and not a single complaint has been received with regard to its use. Returns have been received for 19,328 vaccinated hoggs; of these, 145 died of braxy (0·75 per cent.). On farms where controls have been kept, returns have been received for 3,588 vaccinated hoggs; of these 73 died from braxy (0·8 per cent.). Out of 1,886 controls not vaccinated, 158 died of braxy (8·4 per cent.).

The vaccine is prepared by growing *Cl. septique* on horse flesh broth to which 0·5 per cent. of sterile glucose is added. Growth is for a period of 15 hours at 37°C. Each batch of vaccine is tested for its toxin content by intravenous inoculation of mice, and should be lethal in a dose of 0·025 c.c. An example of such a test is shown in Table I.

TABLE I

TITRATION OF *Cl. septique* TOXIN

Two mice used at each dose—injections intravenously.

TOXIN DOSE.	RESULT.	
0·025 c.c.	+	+
0·01 c.c.	+	L
0·0075 c.c.	L	L
0·005 c.c.	L	L

+ = Died. L = Lived.

The specificity of the toxin is then determined by mixing graded doses of septique antitoxin with a fixed dose of toxin; these mixtures are allowed to stand for one hour, and mice are then inoculated. Table II is an example of such a titration, and shows that the toxin is highly specific.

TABLE II

TEST FOR THE SPECIFICITY OF *Cl. septique* TOXIN USING A STANDARD ANTITOXIN

Two mice used at each dose—injections intravenously.

ANTITOXIN DOSE.	TOXIN DOSE.	RESULT.	
0·00075 c.c.	0·1 c.c.	+	+
0·001 c.c.	0·1 c.c.	+	+
0·0025 c.c.	0·1 c.c.	L	L
0·005 c.c.	0·1 c.c.	L	L

Neutral point—0·0025 c.c. of standard antitoxin neutralises 0·1 c.c. of toxin, showing that the toxin is highly specific.

Toxin and serum mixtures allowed to stand one hour before inoculation.

+ = Died. L = Lived.

0·5 per cent. of formalin (40 per cent. formaldehyde) is added to the culture, and the *p*H adjusted to 7·5. Incubation at 37°C. is carried out for two days, when the vaccine should be non-toxic when inoculated intravenously into

mice in a dose of 0·5 c.c., and non-pathogenic for guinea-pigs by intramuscular injection of 1·0 c.c. Sterility test cultures are made at this stage, and these should remain sterile when incubated for five days at 37°C. The vaccine is then tested for its power to immunise rabbits against a fatal dose of *Cl. septique* toxin, and an example of such a test is shown in Tables III and IV.

TABLE III

TITRATION OF A STANDARD DRY *Cl. septique* TOXIN
Rabbits used—injections intravenously.

TOXIN DOSE.	RESULT.
0·15 c.c.	+ + + +
0·125 c.c.	+ + + + +
0·1 c.c.	L L L + + + +
0·075 c.c.	L L L L L +

The lethal dose of standard toxin selected for test purposes is 0·125 c.c.
+ = Died. L = Lived.

TABLE IV

PROTECTIVE VALUE OF *Cl. septique* FORMALINISED WHOLE CULTURE

Twelve rabbits each inoculated subcutaneously with 5·0 c.c. of vaccine.
Two doses given at 14 days' interval.
Tested 14 days after the second injection by intravenous inoculation of standard *Cl. septique* toxin.

VACCINATED RABBITS.	ONE LETHAL DOSE OF TOXIN.	THREE LETHAL DOSES OF TOXIN.	FOUR LETHAL DOSES OF TOXIN.	EIGHT LETHAL DOSES OF TOXIN.	DEGREE OF PROTECTION IN TERMS OF LETHAL DOSES.
1 ...	+ ...	...	...	...	< 1
2 ...	L ...	+ ...	...	...	1 < 4
3 ...	L ...	+ ...	...	...	1 < 4
4 ...	L ...	+ ...	...	...	1 < 4
5 ...	L ...	L ...	+ ...	...	4 < 8
6 ...	L ...	L ...	+ ...	...	4 < 8
7 ...	L ...	L ...	L ...	+ ...	8 < 16
8 ...	L ...	L ...	L ...	+ ...	8 < 16
9 ...	L ...	L ...	L ...	+ ...	8 < 16
10 ...	L ...	L ...	L ...	L ...	> 16
11 ...	L ...	L ...	L ...	L ...	> 16
12 ...	L ...	L ...	L ...	L ...	> 16
Control Rabbits not Vaccinated.					
1 ...	+ ...	...	...	...	
2 ...	+ ...	...	...	...	
3 ...	+ ...	...	...	...	

+ = Died. L = Lived.

An interval of a few hours elapses between one set of injections and the next, but all injections are completed within 48 hours.

Finally, all batches of vaccine are blended so that a uniform product is issued to all users, and as a final precaution, the vaccine is inoculated subcutaneously into one hundred sheep to ensure its safety before issue.

I have described this method in some detail to show the degree of accuracy with which a veterinary biological product can be standardised, and to impress upon you the precautions which have to be taken to ensure that a standard vaccine is issued year after year.

The Control of Lamb Dysentery

The work of Dalling, Mason and Gordon[7, 8, 9, 10, 11] provides ample evidence that dysentery in lambs can be prevented by two methods:—

(*a*) Passive immunisation by subcutaneous inoculation of lambs with antitoxin on the day of their birth.

(*b*) Active immunisation of ewes by subcutaneous inoculation of formalinised whole culture.

This latter method was adopted for use on hill farms where difficulties were experienced in finding all the lambs born on the day of their birth. By experimental methods it was shown that ewes actively immunised against lamb dysentery do not transmit antitoxin to their lambs through the placenta, but that antitoxin is absorbed by the newly-born lamb from the colostrum of its immune mother; that is to say, the lamb receives a passive immunity.

I do not propose to discuss the standardisation of lamb dysentery vaccine. The principle underlying the process is similar to that which I have outlined in the standardisation of braxy vaccine.

In standardising the antitoxic value of lamb dysentery serum we have adopted the methods employed by the Wellcome Research Laboratories. A unit of antitoxin may be defined as that amount of serum which will neutralise a test dose of toxin, and a test dose of toxin contains many mouse fatal doses. The titration of

a standard serum against the test dose of toxin is shown in Table V, and we know by practical experience that 5 c.c. of such a serum is capable of protecting a lamb against infection with lamb dysentery.

TABLE V

TITRATION OF STANDARD LAMB DYSENTERY ANTITOXIN

Mice used—injections intravenously.

	TEST TOXIN.			
Standard Lamb Dysentery Antitoxin.		Dried Lamb Dysentery Toxin dissolved in Saline.		Result.
0·0006	...	0·1 c.c.	...	+ +
0·0007	...	„	...	+ +
0·0008	...	„	...	L L
0·0009	...	„	...	L L

0·0008 c.c. serum neutralises a test dose of toxin, *i.e.*, 1 unit of serum is equivalent to 0·0008 c.c.,

$\therefore$ 1·0 c.c. of serum contains $\frac{1}{0{\cdot}0008}$ units per c.c.

= 1,250 units per c.c.

+ = Died. L = Lived.

In Table VI is shown the result obtained with a serum issued by a commercial firm when titrated against the same toxin.

TABLE VI

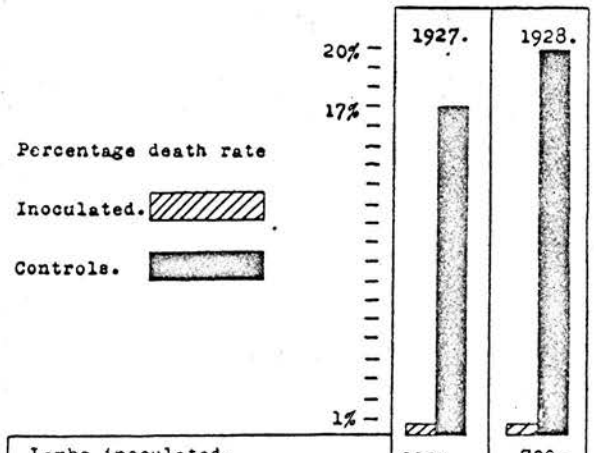
TITRATION OF A LAMB DYSENTERY SERUM ISSUED BY A COMMERCIAL FIRM

	TEST TOXIN.			
Serum.		Dried Lamb Dysentery Toxin dissolved in Saline.		Result.
0·005 c.c.	...	0·1 c.c.	...	+ +
0·006 c.c.	...	„	...	+ +
0·007 c.c.	...	„	...	+ +
0·008 c.c.	...	„	...	+ +
0·009 c.c.	...	„	...	+ +
0·01 c.c.	...	„	...	+ +
0·025 c.c.	...	„	...	+ +
0·05 c.c.	...	„	...	+ +
0·075 c.c.	...	„	...	+ +
0·1 c.c.	...	„	...	+ +
0·2 c.c.	...	„	...	+ +

No detectable lamb dysentery antitoxin.
Unit value nil.
+ = Died.

FIG. 2.

Control of Lamb Dysentery by the inoculation of lambs with antitoxin.



	1927.	1928.
Lambs inoculated.	1122.	709.
Deaths from lamb dysentery.	5.	4.
Control lambs.	1241.	648.
Deaths from lamb dysentery.	213.	136.

The result clearly shows that the serum was non-specific and quite incapable of neutralising lamb dysentery toxin. Such a serum is valueless in the prevention of the disease, it is harmful to the reputation of the veterinary surgeon who uses it, and it brings a reliable biological product into disrepute. To show the value of control measures for preventing lamb dysentery, I have abstracted some of the results obtained in 1927 and 1928, Figs 2 and 3.

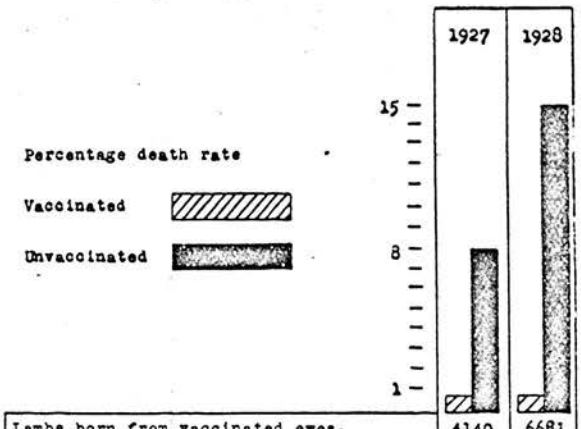
Provided that properly standardised products are used, and the natural conditions of infection remain unaltered, results of this description should be maintained.

The Control of Louping-ill (An Encephalomyelitis of Sheep)

Pool, Brownlee and Wilson[12] demonstrated the presence of the infective agent of louping-ill in the central nervous system of affected sheep. Subsequent work at the Animal Diseases

FIG. 3.

Active immunisation of ewes for the prevention of dysentery in their lambs.



	1927	1928
Lambs born from vaccinated ewes.	4140	6681
Deaths from Lamb Dysentery.	36	59
Lambs born from unvaccinated ewes.	2213	5029
Deaths from Lamb Dysentery.	173	761

Research Association[13, 14, 15] showed that the disease was due to a filtrable virus possessing neurotropic characters. It was also shown that virus could be detected in the blood of affected sheep prior to infection of the central nervous system. This fact explains how it is possible for ticks to acquire infection and so act as vectors of a neurotropic virus. Detailed experiments of this work have already been published, but Fig. 4 depicts the method adopted for experimental infection of ticks.

ICR = Inoculated intracerebrally with louping-ill virus.

\+ = Death due to louping-ill.

0 = No infection.

T.L.I. = Typical louping-ill.

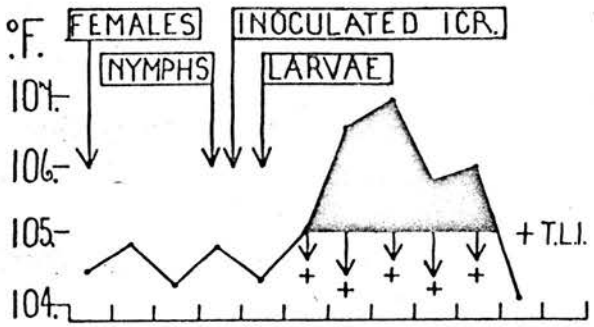
The arrows on the underside of the temperature curves indicate withdrawal of blood for intracerebral inoculation of mice.

In considering methods for the control of louping-ill by immunisation we learned at an early stage in our investigations that the central nervous system was a difficult tissue to protect,

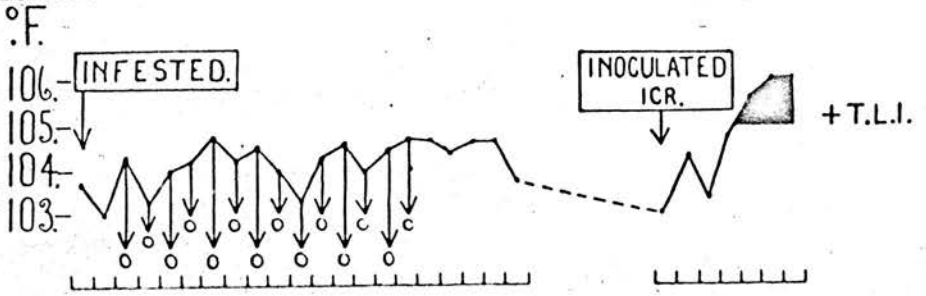
FIG. 4.

METHOD OF INFECTING TICKS.

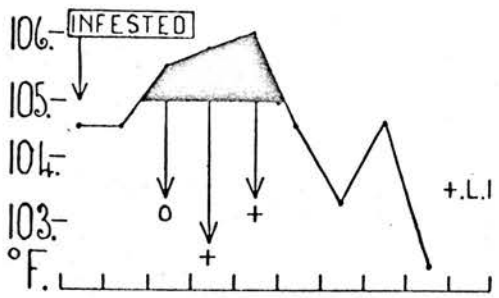
HOST SHEEP.



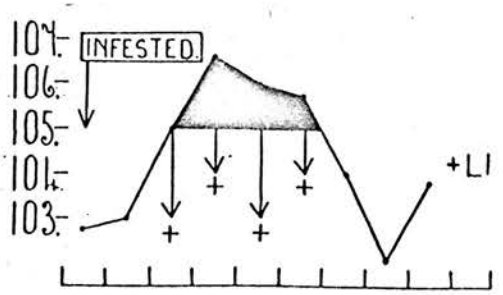
INFESTATION WITH LARVAE BRED FROM FEMALES WHICH FED ON HOST SHEEP.



INFESTATION WITH NYMPHS BRED FROM LARVAE WHICH FED ON HOST SHEEP.



INFESTATION WITH FEMALES BRED FROM NYMPHS WHICH FED ON HOST SHEEP.



The three stages in the life cycle of the tick require different periods of time for engorgement.

Larvæ about three to four days.
Nymphs about five to six days.
Females about eight to nine days.

FIG. 5.

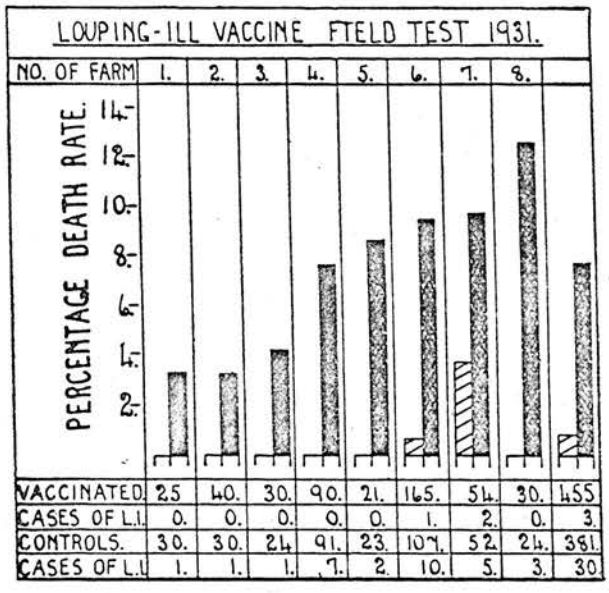

LOUPING-ILL VACCINE FTELD TEST 1931.
NO. OF FARM
1.
2.
3.
4.
5.
6.
7.
8.
PERCENTAGE DEATH RATE.
VACCINATED. 25 40. 30. 90. 21. 165. 54. 30. 455
CASES OF L.I. 0. 0. 0. 0. 0. 1. 2. 0. 3.
CONTROLS. 30. 30. 24 91. 23. 107. 52. 24. 381.
CASES OF L.I 1. 1. 1. 7. 2. 10. 5. 3. 30.

FIG. 6.

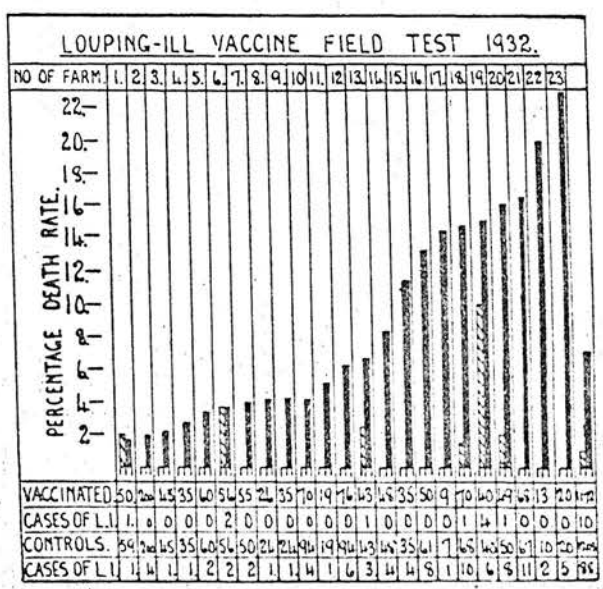
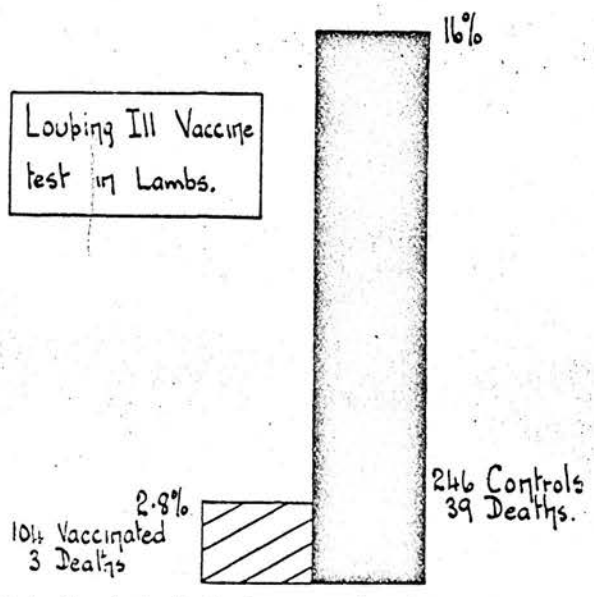

LOUPING-ILL VACCINE FIELD TEST 1932.
NO OF FARM.
PERCENTAGE DEATH RATE.
VACCINATED 50 20 45 35 60 54 55 21 35 70 19 76 43 46 35 50 9 70 40 47 55 13 20 11
CASES OF L.I. 1 0 0 0 0 2 0 0 0 0 0 0 1 0 0 0 0 1 4 1 0 0 0 10
CONTROLS. 59 20 45 35 60 56 50 21 21 98 19 98 43 45 35 61 7 65 43 50 61 10 20 [illegible]
CASES OF L.I. 1 4 1 1 2 2 2 1 1 4 1 6 3 4 4 8 1 10 6 5 11 2 5 88

FIG. 7.



but the fact that virus can be detected in the blood at an early stage of the infection led us to believe that if sufficient immunity were produced to neutralise the virus in the blood, then the central nervous system would not become infected. An experimental vaccine for this purpose has been prepared; the methods of production and standardisation will be published later, but it is appropriate here to show the results of field trials with this vaccine in 1931 and 1932 (Figs. 5, 6 and 7).

This season we have vaccinated about 20,000 sheep, and left about 20,000 controls on the same farms non-vaccinated; the results are encouraging and will be published later.

Tick-borne Fever

The preliminary observations on tick-borne fever have already been published[16, 17]. This is a febrile disease of sheep with a low mortality. It is caused by an infective agent transmitted by ticks, and was discovered during our investigation of louping-ill. A sheep immune to tick-borne fever is still susceptible to louping-ill, and

a sheep immune to louping-ill is still susceptible to tick-borne fever. This disease may yet be shown to be of considerable economic importance to the sheep industry, since we have already obtained evidence to suggest that a sheep affected with this disease has its resistance so lowered that it is liable to develop secondary infections which ultimately cause death. At the present time experiments are in progress to determine if this disease can be controlled.

It is appropriate to mention here that louping-ill and tick-borne fever could also be controlled if a practicable method of abolishing ticks were evolved. This is discussed in a paper which will shortly be published, and in which it is shown that control of the tick population within a fenced enclosure of a diseased farm was followed by the disappearance of these tick-borne diseases, whilst both diseases occurred in their usual incidence on the tick-infested pastures outside the fenced enclosure.

Anærobic Infections

The anærobic infections of sheep have received considerable attention within recent years. On the one hand, this may be due to the fact that under natural conditions of infection sheep are particularly susceptible to infection with anærobic organisms. On the other hand, it may be due to the fact that the anærobic infections of sheep have received closer study than have similar infections of other species of animals. It may yet be shown that some of the obscure diseases of cattle and horses, characterised by their sudden onset, acute and distressing illness and the rapidity with which death occurs, are due to anærobic infections. In the case of braxy, as it occurs in Scotland, *Cl. septique* invades the stomach wall, and is often found in the heart blood of affected animals. Lamb dysentery is caused by invasion of the intestinal wall by a type of *Cl. welchii*, referred to as the " agni " type. The organism in this case appears to be less invasive than *Cl. septique*, since it has been found in the heart blood of only a small proportion of cases. Black disease in Australia[15] has been determined as an infection of the liver with *Cl. œdematiens*; this organism also has

very low invasive powers, since it apparently remains confined to the liver, where toxin presumably responsible for death of the animal is produced. " Struck," a disease of sheep on the Romney Marsh, described by McEwen and Roberts[19], is caused by a type of *Cl. welchii*, referred to as the " paludis " type. The main lesion of this disease is an enteritis, often acute, and accompanied by ulceration. Though the causal organism often invades the tissues, cases occur in which tissue invasion cannot be demonstrated. In such cases it has been shown that *Cl. welchii* toxin of the " paludis " type can be detected in the intestine. Bennetts[20] has described a disease of sheep in Western Australia, infectious entero-toxæmia, caused by a type of *Cl. welchii*, referred to as the " ovitoxicus " type. This disease is a toxæmia quite independent of any tissue invasion by the causal organism, although a few organisms may be found in blood and tissues at the point of death. Gill[21] has shown that pulpy kidney disease of lambs is due to a similar, if not identical, organism. Oxer[22] has described the same disease in Australia; Montgomery and Dalling[23] record the finding of a toxin resembling that described by Bennetts, in the intestine of lambs in this country which had died of pulpy kidney disease, and in ewes which had died of " struck." After studying a number of different strains of *Cl. welchii*, Wilsdon[24] recognised four types which he referred to as A, B, C and D. A appears to be the classical type of *Cl. welchii* isolated from cases of gas gangrene in man; B, *Cl. welchii* of the " agni " type isolated from cases of dysentery in lambs; C, *Cl. welchii* of the " paludis " type isolated from cases of " struck " on the Romney Marsh, and D, a type which had hitherto not been recognised. Work at the Wellcome Laboratories[25] suggests that Wilsdon's type D is similar to, if not identical with, Bennetts' *Cl. welchii* of the " ovitoxicus " type.

The control of the various diseases of sheep caused by these anaerobes is in some instances only in its experimental stage, but when one realises the effectiveness of prophylactic vaccines in the prevention of braxy and lamb dysentery,

there is every reason for confidence that effective measures for the control of most of the anærobic infections of sheep will eventually be evolved.

This brief survey gives some idea of the complexity of the problem of anærobic infections and the wide field of possibilities which their study has opened up. In this connection I would mention that we have undertaken an investigation of the anærobic flora of the bowel and bowel contents of horses affected with grass disease, and in our preliminary studies have detected the presence of *Cl. welchii* toxin of the "ovitoxicus" type in the small intestine of one acute case of this disease. In another case, although this toxin was not detected in the bowel contents, the presence of the organism has been demonstrated. The experimental details of this work will be published later, but, taking into consideration the low invasive powers of this organism and the previous more or less negative bacteriological findings in connection with grass disease in horses, I venture to suggest that this line of enquiry, which we intend to investigate fully, may yet prove helpful in the elucidation of this baffling disease problem.

[In concluding, Mr. Gordon said he wished to take that opportunity of thanking Dr. Greig for the way in which he facilitated their work, and he wished especially to thank his colleagues, Mr. Brownlee, Mr. Wilson, and Dr. MacLeod, for their sterling work.]

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A CONSIDERATION OF METHODS FOR THE CONTROL OF LOUPING-ILL

Since louping-ill is caused by a filtrable virus which is transmitted by the bite of infective ticks, alternative measures for the control of the disease present themselves for consideration:-

(1) Control of the disease by reducing or abolishing the tick population on a "diseased" farm, thereby limiting the spread of infection from diseased to susceptible animals.

(2) Control of the disease by immunisation of the sheep against infection transmitted by the ticks, which, besides limiting the number of sheep infected, might eventually have the effect of cleansing infection from the ticks.

No practicable method of exterminating ticks has so far been devised; consequently, hope of controlling the disease seemed to lie in the alternative measure of prophylactic vaccination, provided a suitable vaccine could be evolved. In order to determine, under field conditions, the comparative merits of the principles involved in these control measures, and for the purpose of obtaining direct evidence of the existence of abortive cases of louping-ill, a second field experiment was undertaken. Since the first field experiment had revealed the prevalence of tick-borne fever on tick-infested farms, this disease had also to be considered, and the second

experiment/

experiment was arranged so that information regarding the natural occurrence and effects of tick-borne fever might also be obtained.

A FIELD EXPERIMENT - 1932

Preparation of the Experimental Pasture

In 1929, Mr. W. A. Pool, then Director of the Moredun Institute, initiated a scheme for de-ticking a ten-acre area of a tick-infested farm where louping-ill was known to occur. This area was fenced with a double fence, and a space of 45 yards broad was left between the inner fence and the outer; the latter had a 1½ inch mesh to prevent access of ground game. In May, 1929, sheep were dipped and placed within the central enclosure. While grazing on this pasture they were dipped every fifth day in an arsenic dip. In June the sheep were removed and the plot left vacant. In September of the same year, forty-two sheep were dipped and carried into the central enclosure. While grazing within the plot they were dipped every third day in an arsenical bath. After six dippings they were removed. In September of the following year, twenty-six sheep were grazed on the plot and dipped as previously for five successive dippings. At the commencement of this period some ticks were found infesting the sheep, but at the

end/

end of the period the sheep were no longer collecting ticks. The plot was then left vacant until April, 1932, when the present experiment was arranged. Thus, for the purpose of the field experiment there was available a ten-acre fenced area of hill pasture on which it was hoped that the tick population had been reduced or abolished, and outside the enclosure the same type of pasture on which the normal tick population kept alive by feeding on the native stock remained undisturbed.

Preparation of the Experimental Sheep

Five groups of sheep were prepared for the experiment, and comprised the following:-

Group I

Twenty sheep believed to be susceptible to louping-ill and tick-borne fever. These animals were grazed outside the enclosure where ticks were prevalent. It was anticipated that they would become infested with ticks; that all would develop tick-borne fever; and that a certain percentage would become infected with louping-ill.

Group II

Ten sheep believed to be susceptible to louping-ill and tick-borne fever. These animals were carried into the fenced enclosure and grazed within it. Since the/

tick population within the enclosure had been controlled, it was hoped that the sheep grazed on this pasture would not develop either of the tick-borne diseases.

Group III

Ten sheep immunised against tick-borne fever. These animals were immunised against tick-borne fever, by repeated subcutaneous inoculation with infective blood, until they failed to react. They were grazed on the pasture outside the enclosure where the normal tick population existed. It was anticipated that a certain percentage of these animals would develop louping-ill infection without the complicating factor of a concurrent infection with tick-borne fever.

Group IV

Ten sheep rendered immune to louping-ill by subcutaneous inoculation with a dead vaccine (discussed later in this paper), followed by living virus subcutaneously. Although some of these animals were not immune to louping-ill virus introduced intracerebrally, all were proved to be immune to virus introduced into the general circulation. These sheep were grazed on the tick-infested pasture, and were expected to develop tick-borne fever without/

without the complication of a louping-ill infection.

Group V

Ten sheep immunised against both louping-ill and tick-borne fever. These animals were immunised against louping-ill by subcutaneous inoculation with living virus, and were proved to be immune. Following this course of immunisation, the same animals were infected with tick-borne fever a number of times till finally they failed to react when virulent blood was injected. They were grazed on the tick-infested pasture, and it was anticipated that they would not develop either of the tick-borne diseases.

Nature of the Observations made throughout the Experiment

The five groups of sheep were transferred to the experimental farm on April 12th, and returned to the laboratories on June 3rd. In order that they would not suffer hardship from lack of food, the animals were supplied daily with a full ration of artificial food. During the experiment the animals were kept under observation, and the following procedure adopted:-

(1) The temperature of every sheep was recorded daily.

(2) As an index of the degree of tick infestation, a count of the nymphal ticks on the head and ears of the sheep was made at frequent intervals.

(3)/

(3) For the purpose of detecting abortive cases of louping-ill, a blood sample was drawn from each animal whose temperature reached 106°F, or over. These samples of blood were inoculated intracerebrally into mice, which were kept under observation for not less than twenty days.

(4) Any animal which became so debilitated that death seemed imminent was destroyed, and all animals in this category, and those found dead, were dealt with in the following manner. A complete post mortem examination was carried out and representative portions of the central nervous system were removed for histological examination. Pieces of the brain, cervical, dorsal and lumbar cord were emulsified together in a mortar, and saline added to make a 10 per cent. suspension. A similar emulsion was made from the spleen and pancreas separately, and two mice were inoculated intracerebrally with 0.05 c.c. of each suspension.

(5) The sheep which survived exposure to natural infection were returned to the laboratories and tested along with suitable controls, firstly for immunity to tick-borne fever and, secondly, for immunity to louping-ill.

This series of observations permitted a study of the nature of the febrile reactions which occurred in each group of sheep. The result of the tick eradication scheme on the tick population within the fenced enclosure was assessed by comparative counts of the nymphal ticks observed on the head and ears of the sheep within the enclosure and those observed on the sheep outside the enclosure. The inoculation of mice, with blood drawn from

sheep/

sheep affected with a febrile reaction, detected those animals in which louping-ill virus was present in the blood. If a sheep died, or was destroyed, an opinion as to whether or not it had been affected with louping-ill was formed, firstly as a result of intracerebral inoculation of mice with selected tissues, and, secondly, as a result of histological examination. Abortive cases of louping-ill were determined by the detection of virus in the blood of animals which developed a febrile reaction not followed by death, and further confirmation of this diagnosis was obtained by testing the sheep after recovery for immunity to louping-ill. Finally, the incidence of tick-borne fever was estimated by means of the immunity test to determine if the surviving animals had acquired immunity to this disease. The result of these observations has been assembled in five figures. Each figure depicts the phenomena which occurred in the individual animals of each group, and the effects in each of the five groups will now be considered separately.

Group I

Phenomena which occurred in Sheep, susceptible to Louping-ill and Tick-borne Fever, when grazed on the Tick-infested Pasture of a Louping-ill Farm

Fig. V, left-hand column of temperature charts, details/

details the nature of the febrile reactions which occurred in the animals of this group during exposure to natural infection. The blackened areas of the charts indicate periods in the febrile phase when temperatures above 105°F were recorded. The arrows on the under-side of the curves indicate the days on which blood was drawn from the reacting sheep and inoculated intracerebrally into mice. Where the sign + is inserted at an arrow head, it means that the mice inoculated developed typical louping-ill. The sign ± indicates that the inoculated mice died from undetermined cause within a louping-ill incubation period. In such cases the brain of the dead mouse was removed, emulsified in saline and inoculated into other mice, always with a negative result. These confirmatory inoculations were often difficult owing to the contaminated nature of the material obtained from some of the dead animals. Whilst, in the majority of those cases, it is probable that the virus of louping-ill was not present in the material examined, it is possible that in a few instances this virus may have been present, although it was not detected by the methods adopted. The result of this section of the experiment may be outlined as follows:-

(1)/

(1) Within ten days every sheep developed a febrile reaction. In most cases the febrile period was very prolonged; thus, in Sheep No. 519 it lasted for nearly two months.

(2) During the two months' period of the experiment there was a 60 per cent. mortality. Seven of the animals died, and five were destroyed at the point of death.

(3) The virus of louping-ill was detected in the blood of seven animals (Nos. 504, 509, 511, 513, 515, 519 and 520) during a febrile reaction. Four of these (Nos. 504, 511, 513 and 520) died, or had to be destroyed. Three survived (Nos. 509, 515 and 519) and did not develop symptoms of louping-ill infection. This establishes the existence of abortive cases of louping-ill in which virus is present in the blood during a febrile reaction which is not followed by symptoms indicative of central nervous system involvement.

(4) The virus of louping-ill was detected in the tissues of the central nervous system, spleen and pancreas of two sheep (Nos. 511 and 520), which died. Prior to death neither of these animals were seen showing symptoms diagnostic of louping-ill.

(5) Histological examination of tissues from the animals which died or were destroyed revealed the presence of definite lesions of louping-ill in the central nervous system of three (Nos. 513, 518 and 520). Indefinite lesions, but lesions which could be associated with the activity of louping-ill virus in the central nervous system and its coverings, were found in two sheep (Nos. 507 and 511).

Test of the Surviving Animals for Immunity to Tick-borne Fever

The second column of temperature charts in

fig./

fig. V depicts the result of testing the eight surviving sheep for immunity to tick-borne fever. This test consisted in the subcutaneous inoculation of the animals with 5.0 c.c. of blood obtained from a sheep infected with tick-borne fever. A normal sheep was also inoculated with the same test material, and the temperature chart of the control animal appears immediately above the chart of the tested sheep. It will be seen that all the animals had acquired immunity to tick-borne fever as a result of exposure to natural infection; in some the immunity was complete; in others, an abortive reaction followed the test dose. This result, coupled with the nature of the febrile reactions which occurred in most of the animals while grazing on the tick-infested pasture, suggested that tick-borne fever occurred in every animal except No. 502, which died on the seventh day after the commencement of the experiment.

Test of the Surviving Animals for Immunity to Louping-ill

Following the test for immunity to tick-borne fever, the sheep were tested for immunity to louping-ill by intracerebral inoculation with the specific virus. Suitable controls were inoculated at the same time with a similar dose of virus to control the infectivity/

infectivity of the test material. The temperature chart of each control animal appears immediately above the chart of the tested sheep. The result is shown in fig. V, from which it will be seen that three of the eight survivors (Nos. 509, 515 and 519) were immune to louping-ill, and reference to the first column of charts in the same figure shows that the virus of louping-ill was detected in the blood of those animals during the natural febrile infection. In the case of the four animals which were not immune, the virus of louping-ill was not detected in the blood at any stage during the period of exposure to natural infection.

Inference: When sheep susceptible to tick-borne fever and louping-ill are transferred to the tick-infested pasture of a louping-ill farm, one hundred per cent. of the animals develop tick-borne fever, and about fifty per cent. also become infected with louping-ill. As a result of these infections as many as sixty per cent. of the freshly-introduced animals may die within two months. This mortality probably represents the so-called acclimatisation mortality of tick-infested hill farms.

Comparison of the Degree of Tick Infestation of the Sheep within the Fenced Enclosure and of those outside the Enclosure

Before considering the results in the sheep of Group II, which were grazed inside the fenced enclosure/

enclosure within which tick eradication had been attempted, it is appropriate first to compare the tick population inside and outside the enclosure. This comparison is shown graphically in fig. VI. Thus, inside the enclosure the average count of nymphal ticks on the head and ears of the sheep was never higher than 5.3, whereas outside the enclosure the average count reached a height of 45 nymphal ticks per sheep. It was surprising that so many ticks were still alive within the enclosure, but in the opinion of my colleague, Dr. J. MacLeod, the ticks present must have been kept alive by feeding on small rodents and birds, and could not in their previous stage have fed on sheep.

Group II

Phenomena which occurred in Sheep, susceptible to Louping-ill and Tick-borne Fever, when grazed on the Pasture of a Louping-ill Farm on which the Tick Population had been controlled

Fig. VII depicts the result obtained by grazing susceptible sheep on the same farm, but within the fenced enclosure in which the tick population had been controlled. The left-hand column of temperature records shows a marked difference from those of fig. V. A definite febrile reaction was observed in

three/

three sheep only (Nos. 462, 463 and 478). The reaction in these animals occurred later, and was much less prolonged than the reactions which occurred in the sheep of Group I. During these febrile reactions the virus of louping-ill was not detected in the blood of the reacting sheep. It is believed that the ~~three~~ animals which reacted were infected with tick-borne fever; but it has not been definitely ascertained by what means they became infected. That the febrile reactions were due to tick-borne fever infection was proved by inoculation of normal sheep and sheep immune to tick-borne fever with blood drawn during the febrile phase from two of the reacting animals. The normal animals developed tick-borne fever, whilst the immune animals did not react. The most significant feature of this section of the experiment, however, was the fact that none of the animals in this group died.

Test of the Surviving Animals for Immunity to Tick-borne Fever

Reference to the second column of temperature charts shows that although very mild febrile reactions occurred in the sheep of Group II while they grazed on the experimental farm, most of them, when tested, showed some degree of immunity to tick-borne fever./

fever. In seeking an explanation of this result, it was subsequently found that although these sheep had been purchased in a tick-free district, they had been imported into this district from an area in which ticks are known to be prevalent on some of the farms; consequently, as lambs they may have been infected with tick-borne fever. This would account for an immunity to this disease, which otherwise is difficult to explain.

Test of the Surviving Animals for Immunity to Louping-ill

Since the virus of louping-ill had not been detected in the blood of any of the animals of this group while they grazed within the enclosure, it was anticipated that all would be susceptible to louping-ill. Following the immunity test for tick-borne fever the sheep were inoculated intracerebrally with louping-ill virus. Fig. VII depicts the result, from which it will be seen that none of the animals were immune.

Inference: If the ticks present on a louping-ill farm were exterminated, non-acclimatised sheep could be introduced to the pasture with a comparative degree of assurance that they would not develop louping-ill or tick-borne fever, and the mortality incidence would, in all probability, be low.

Group III

Phenomena which occurred in Sheep, susceptible to Louping-ill but immune to Tick-borne Fever, when grazed on the Tick-infested Pasture of a Louping-ill Farm

Fig. VIII depicts the result. Febrile reactions of short duration occurred in most of the sheep. Some of these reactions may have been abortive infections with tick-borne fever, but in a number of instances the reactions were due to infection with louping-ill. Louping-ill virus was detected in the blood of five of the ten sheep (Nos. 328, 343, 344, 357 and 360). Two animals died - a mortality of twenty per cent. One of these (No. 343) was destroyed when showing typical symptoms of louping-ill, and this was the only animal in any group which showed definite symptoms diagnostic of louping-ill. In this animal the virus of louping-ill was detected in the blood prior to death, and definite lesions of louping-ill were present in the nervous system, but the specific virus was not detected in the central nervous system, spleen or pancreas. The other animal (No. 346) died suddenly, and although louping-ill virus was not detected in its tissues, the lesions in the nervous system suggested that it had been affected with louping-ill.

Test/

Test of the Surviving Animals for Immunity to Tick-borne Fever

Although the animals of Group II had been immunised against tick-borne fever, they were tested after their return to the laboratories and, as anticipated, all were found to be immune. Fig. VIII, second column of temperature charts, depicts the result.

Test of the Surviving Animals for Immunity to Louping-ill

Of the eight sheep in this group which survived exposure to natural infection, the virus of louping-ill had been detected in the blood of four (Nos. 328, 344, 357 and 360). When tested by intracerebral inoculation with the specific virus, these animals were found to be immune. The virus of louping-ill had not been detected in the blood of the four remaining animals, and they were still susceptible to louping-ill when tested for immunity. The last column of temperature charts in fig. VIII depicts the result.

Inference: From this section of the experiment, it may be concluded that it is possible, by artificial methods, to render sheep comparatively immune to a natural attack of tick-borne fever. Such animals/

animals are still susceptible to louping-ill and may develop this infection. Whilst the number of animals in this group is small, the evidence suggests that when sheep are immune to tick-borne fever they are less liable to die as a result of natural infection with louping-ill virus, though a twenty per cent. mortality may occur.

Group IV

Phenomena which occurred in Sheep, susceptible to Tick-borne Fever but immune to Louping-ill when grazed on the Tick-infested Pasture of a Louping-ill Farm

The result of this phase of the experiment is depicted in fig. IX. The febrile reactions which occurred in the animals of this group during their period of exposure to natural infection were due to tick-borne fever. In no case was the virus of louping-ill detected in the blood of any of the animals during the febrile reactions which occurred. The degree of reaction in some of the animals was not very severe. These sheep were fellows to those in Group II, and, as already explained, they may have had some degree of immunity to tick-borne fever as a result of a previous infection. One of the animals (No. 475) died - a mortality of 10 per cent. The nature of the febrile affection in this animal suggests that the cause of death was a primary infection/

infection with tick-borne fever, followed by pneumonia and pleurisy as a secondary complication.

Test of the Surviving Animals for Immunity to Tick-borne Fever

The nine surviving animals were found to have a considerable degree of immunity to tick-borne fever. The result of these tests is depicted in the second column of temperature charts in fig. IX.

Test of the Surviving Animals for Immunity to Louping-ill

Prior to exposure to natural infection, the ten animals in Group IV were immunised against louping-ill by means of subcutaneous inoculation with dead vaccine followed by living virus subcutaneously. Of the nine survivors, four were immune to intracerebral inoculation with louping-ill virus, and five were not immune. Although not immune to intracerebral inoculation with louping-ill virus, all were known to be immune to virus introduced into the general circulation. This phenomenon will be discussed in detail in the section dealing with immunity.

Inference: It may be concluded, as a result of this experiment, that it is possible, by artificial methods,/

methods, to render sheep immune to a natural attack of louping-ill. Such animals may still develop tick-borne fever, but the mortality in all probability will be low.

Group V

Phenomena which occurred in Sheep, immune to both Louping-ill and Tick-borne Fever, when grazed on the Tick-infested Pasture of a Louping-ill Farm

Fig. X depicts the result. All the animals in this group survived exposure to natural infection. One animal only (No. 986) developed a febrile reaction. The nature of this reaction was not definitely ascertained. It was not due to louping-ill infection, since the specific virus was not detected in the blood during the febrile phase; it may, however, have been due to tick-borne fever, since it has previously been found that considerable difficulty is sometimes experienced in immunising certain sheep against this disease. Sheep No. 986 probably falls into this category, as, even after a course of immunisation, followed by exposure to natural infection, this animal still developed a febrile reaction when tested for immunity to tick-borne fever after its return from the experimental farm.

Test for Immunity to Tick-borne Fever

The ten animals in this group were immune to tick-borne fever, with the exception of No. 986, which developed a considerable reaction when tested with virulent blood (see fig. X).

Test for Immunity to Louping-ill

Since all the animals of this group were originally immunised against louping-ill by means of a subcutaneous inoculation with living virus, all were found to be immune to louping-ill when tested by intracerebral inoculation with the specific virus (see fig. X).

Inference: It may be concluded, as a result of this experiment, that non-acclimatised sheep can be acclimatised by artificial methods, so that they may be introduced into a tick-infested hill pasture without the risk of the usual mortality which accompanies the natural acclimatisation process.

Discussion

As a result of this field experiment, it may be concluded that louping-ill and tick-borne fever can be controlled by alternative methods. Thus, on the one hand, if the tick population of a "diseased" farm is controlled or abolished, the two diseases which are transmitted by the ticks disappear; on the other hand, if the sheep are immunised against the infections transmitted by the ticks, the same result is accomplished. The evidence also suggests that when the two diseases develop in the same animal concurrently, each disease seems/

seems to aggravate the harmful effects of the other. The phenomena which occurred in the animals of Group I when they were grazed on the tick-infested pasture can be taken as an index of what may occur when "non-acclimatised" animals are introduced into a "diseased" farm. Although the death rate amongst the native stock on this farm was negligible during the period of the experiment, a sixty per cent. death rate occurred in the freshly-introduced animals. This "acclimatisation" mortality would appear to be due primarily to tick-borne diseases, with, in some cases, secondary bacterial infections. Experimentation continued on the lines of this field experiment might eventually afford a complete biological interpretation of the meaning of acclimatisation of the sheep to many of the hill farms in Scotland. At the present time it would seem that immunity to the tick-borne diseases is one of the most important factors, and the three surviving animals of Group I which had acquired immunity to tick-borne fever and louping-ill, although in a very debilitated condition at the end of the experiment, might be looked upon as acclimatised animals with a reasonable chance of living to an old age on a diseased farm. At the present time experience with these tick-borne diseases indicates that louping-ill is

a/

a much more serious cause of loss amongst the sheep than tick-borne fever. When the latter disease is transmitted in series from sheep to sheep, the febrile affection produced, although sometimes very prolonged, rarely proves fatal. In view of these facts, it was decided to concentrate on methods of producing immunity to louping-ill, since it was considered that if immunity against this disease were produced, much of the death rate on tick-infested hill farms might be abolished. It now remained to evolve a safe and effective method of prophylactic vaccination, and with this end in view, an immunological study of louping-ill was undertaken.

ACTIVE IMMUNITY TO LOUPING-ILL

Immunisation by Means of Living Virus

As already indicated, if a sheep develops a febrile reaction following subcutaneous inoculation with louping-ill virus and survives the infection, it is immune on recovery to an infective dose of virus inoculated intracerebrally. Fig. XI is typical of an active immunity of the central nervous system produced by this means. This method of producing immunity is attended by a risk of setting up the disease, and after preliminary experiments ("Studies in Louping-ill. I."), the method was finally abandoned as being distinctly unsafe for prophylactic vaccination.

Immunisation by Means of Inactivated Virus

PUNTONI[17,18] showed that it was possible to produce immunity in dogs against dog distemper virus by means of a vaccine prepared from a virus-containing tissue which had been treated with formalin. This work was confirmed by SANSONETTI[20]. LAIDLAW & DUNKIN[10] later established that it was possible to immunise ferrets against dog-distemper by means of vaccines consisting of crude distemper virus inactivated by storage, heat, phenol, or formaldehyde.

The/

The evidence indicated that the effective antigen was a dead virus, and that in order to consolidate the immunity induced by such vaccines, living virus must subsequently be administered. They concluded that the solid immunity thus resulting was of long duration, lasted possibly for life, and that the central nervous system partook in the general immunity. Following these experiments, LAIDLAW and DUNKIN[11] showed that it was possible to immunise dogs against distemper by means of similar vaccines prepared from virus-containing tissues obtained from dogs infected artificially.

Since typical cases of louping-ill in sheep are due to invasion of the central nervous system by the specific virus, it was considered probable that a vaccine capable of immunising animals against natural infection would require not only to produce immunity against virus introduced by the ordinary paths of infection, but also against virus which might gain direct access to the central nervous system, possibly along the olfactory nerves, which is a suggested portal of entry of virus in poliomyelitis infection of Man. The work on distemper prophylaxis was, therefore, a guide in the subsequent experiments on immunisation against louping-ill, but a certain prejudice/

prejudice against the use of living products at any stage for large scale immunisation prompted a full investigation of the possibility of producing immunity by means of inactivated virus in preference to any method involving the use of the living agent.

In the course of preliminary studies on louping-ill, it had been found that the specific virus regularly invades the blood during the early part of the febrile stage, and late in the course of infection, when nervous symptoms are manifest, virus can be detected regularly in the central nervous system. The virus has also been detected fairly constantly in the spleen, with less regularity in the mesenteric glands and, very occasionally, in the liver. Preliminary experiments on the effect of formalin on louping-ill virus determined that the addition of 0.15 per cent. of formalin (40 per cent. formaldehyde) to a virus-containing suspension had the effect of inactivating the virus after contact for four days. Accordingly, an experimental vaccine was prepared from the tissues of the central nervous system and spleen of sheep infected with louping-ill by intracerebral inoculation. After experimentation with various methods, the following technique, which is the one in use at the present time, was finally adopted.

Technique for the Preparation of Louping-ill Prophylactic Vaccine

Sheep are inoculated intracerebrally with louping-ill virus, and between the fifth and seventh day after infection, when nervous symptoms have developed, the animals are destroyed under a general anaesthetic by bleeding. Two lots of vaccine are prepared from each animal; the first is prepared from the brain and spinal cord, and the second from the spleen. These tissues are removed with strict sterile precautions into sterile weighed containers, and the weight of each tissue determined. The tissues are transferred to sterile mortars and pulped without the aid of sand or glass. When the material has the consistency of a smooth paste, saline is added to make a 20 per cent. suspension of the pulped tissue. The whole is then poured into sterile bottles which contain chips of flint, and the bottles are shaken for fifteen minutes in an automatic shaker. The contents are filtered through a double thickness of butter muslin into a sterile bottle. A small quantity of the suspension is removed and tested for potency by intracerebral inoculation of mice. To the remainder 0.25 per cent. formalin solution (40 per cent. formaldehyde) is added, giving a final concentration of formaldehyde of 0.1 per cent.

The/

The mixture is again shaken and transferred to the cold store. After four days samples are withdrawn and sterility tests made. The sterility tests include the inoculation of culture medium to detect bacterial contaminants, and the intracerebral inoculation of mice and sheep to detect the presence of living virus. Finally, the vaccine is tested for antigenic value, and if it conforms to the following requirements, it is issued for prophylactic vaccination:- (a) It must be bacteriologically sterile; (b) it must not cause disease when inoculated intracerebrally into mice and sheep; (c) it must not cause any serious local reaction when inoculated subcutaneously into sheep; (d) it must be capable of protecting sheep against a test dose of virus introduced subcutaneously.

This type of vaccine has been prepared on a large scale, but the detail of the original experiments is as follows:- Sheep No. 24 was inoculated intracerebrally with 1.0 c.c. of a 1 per cent. saline suspension of the brain from a sheep infected with louping-ill. The animal developed a febrile reaction, followed by typical symptoms of louping-ill, and was killed on the fifth day after inoculation. Separate vaccines were prepared in the manner/

manner previously described from the tissues of the central nervous system, spleen, mesenteric glands and liver. The following table gives the details of preparation, and the result of the potency and sterility tests.

"Louping-ill Brain Vaccine," Batch 24

Tissue	Brain	Spleen	Mesenteric Gland	Liver
Weight	80 gms.	24.5 gms	26 gms.	400 gms.
Saline added	320 c.c.	98 c.c.	104 c.c.	1600 c.c.
Formalin added	0.25%	0.25%	0.25%	0.25%

Potency Test - Mice inoculated intracerebrally
Dose: 0.1 c.c. of 1 in 50 tissue suspension in saline.

+ 7	+ 8	+ 8	+operation
+ 7	+10	+ 8	0
	+10	+ 9	0

Sterility Test for Virus - Mice inoculated intracerebrally with 0.1 c.c. of vaccine

0 0	0 0 0 0	0 0	0 0

Sterility Test for Bacteria - Vaccine inoculated into horse flesh broth and blood agar and incubated for seven days.

-	-	-	-

+ x = Death in mice due to louping-ill with an incubation period of x days.
0 = Mice remained healthy.
- = Cultures remained sterile.

The Immunising Value for Sheep of "Louping-ill Brain-Vaccine," Batch 24.

One Inoculation with Vaccine Subcutaneously

Two sheep (Nos. 26 and 32) were inoculated subcutaneously with 20 c.c. of "brain-vaccine," batch 24. Fourteen days later the same animals, along with one normal sheep, were tested for immunity by intracerebral inoculation with 1.0 c.c. of a 1 per cent. saline suspension of dried brain 5, the desiccated and powdered brain of a sheep infected with louping-ill. This material regularly killed sheep in not more than six days after inoculation, Fig. XII shows that the control animal died on the sixth day after inoculation, whilst the two vaccinated animals died of typical louping-ill in nine and eight days respectively. Since the dried brain 5 material regularly killed sheep within six days, the slightly longer incubation period and the nature of the febrile reaction which followed intracerebral inoculation of the two vaccinated animals suggests that they had acquired some degree of immunity to louping-ill. It is generally recognised that it is difficult to immunise the central nervous system against toxins, and it was considered possible that intracerebral inoculation with virus might be an unduly severe and exacting test of immunity, particularly if/

if the central nervous system did not partake in the general immunity. Accordingly, two sheep (Nos. 40 and 41) were inoculated subcutaneously with 20 c.c. of brain vaccine 24. Fourteen days later the same animals, along with a control sheep, were inoculated subcutaneously with 10 c.c. of a 1 per cent. saline suspension of dried brain 5. Fig. XIII depicts the result, from which it will be seen that the two vaccinated animals did not react to the test dose of virus, whereas the control sheep developed a febrile reaction and definite constitutional disturbance. There was, therefore, evidence that although one dose of vaccine did not immunise the central nervous system, it produced sufficient systemic immunity to neutralise virus introduced subcutaneously.

One Inoculation with Dead Vaccine Subcutaneously, followed by Living Virus Subcutaneously

From the analogy of distemper immunisation, it was considered probable that the living virus, inoculated subcutaneously into sheep which had been immunised previously with a dose of vaccine, would consolidate the immunity, and it was anticipated that these two animals used in the previous experiment, would be immune to intracerebral inoculation with/

with virus. Accordingly, fourteen days after the subcutaneous inoculation of living virus, sheep Nos. 40 and 41, along with a normal sheep, were tested for immunity to louping-ill by intracerebral inoculation with the specific virus. The control sheep died on the sixth day after inoculation, and the two immunised animals died of typical louping-ill in seven and eight days respectively. The result of this test is shown in fig. XIV.

This experiment indicates that living virus, introduced subcutaneously into a sheep which has been immunised previously by means of a subcutaneous inoculation with dead vaccine, does not appreciably increase the immunity of the central nervous system. If, however, the same dose of living virus had been inoculated into a sheep not previously treated with dead vaccine, then the central nervous system would have been immunised.

Double Subcutaneous Inoculation with Dead Vaccine

In order to test the immunising value of a double inoculation with dead vaccine, two sheep (Nos. 35 and 36) were inoculated subcutaneously on two occasions with 20 c.c. of brain vaccine, batch 24. An interval of fourteen days elapsed between the first and second inoculation. Fourteen days after/

after the second injection the two animals, along with a control, were tested for immunity by intracerebral inoculation with 1.0 c.c. of a 1 per cent. saline suspension of dried brain 5. The result is depicted in fig. XV. The control sheep died in six days, with symptoms of acute louping-ill, whereas Sheep No. 35 developed a chronic type of louping-ill and died on the tenth day after infection, whilst sheep No. 36 was apparently immune. Although this experiment was carried out in a small number of animals, the evidence suggests that for immunisation of the central nervous system against louping-ill virus, double subcutaneous inoculation with dead vaccine is a more effective method than one dose of dead vaccine subcutaneously, followed by a dose of living virus subcutaneously.

Intracerebral Inoculation with Dead Vaccine

Two sheep (Nos. 33 and 34) were inoculated intracerebrally with 1.0 c.c. of dead vaccine. No reaction followed, and the two animals, along with a control, were tested for immunity fourteen days later by intracerebral inoculation with 1.0 c.c. of a 1 per cent. saline suspension of dried brain 5. The three animals developed typical louping-ill and died in six days. Since only a limited quantity of vaccine/

vaccine can be inoculated intracerebrally, and since the method is not suitable for practical application, further investigation of its value was not undertaken. The spleen, mesenteric gland and liver vaccines prepared from sheep No. 24 were also tested for immunising value in sheep. The spleen was found to be as good an antigen as the brain, but the mesenteric gland and liver vaccines were valueless.

ATTEMPTS TO IMMUNISE MICE AGAINST LOUPING-ILL

All attempts to immunise mice against louping-ill have so far failed. Mice have been given multiple subcutaneous inoculations with living virus, and after as many as six inoculations at intervals of fourteen days the animals were not immune to intracerebral inoculation with louping-ill virus. Dead vaccines prepared from the brains of infected mice, although capable of protecting sheep against subcutaneous inoculation with louping-ill virus, were quite incapable of immunising mice against intracerebral inoculation with virus.

LOUPING-ILL PROPHYLACTIC VACCINE - 1931

Under natural conditions of infection, louping-ill is transmitted by the bite of infective ticks. In all probability, the virus is introduced into the/

the general circulation, and multiplication occurs primarily in the blood. If, on the one hand, the virus fails to gain access to the central nervous system, the animal usually recovers and is immune to subsequent infection; on the other hand, if the virus invades the central nervous system, the typical symptoms of louping-ill develop and the animal generally dies. As this appears to be the most probable explanation of the pathogenesis of the disease, it was considered possible that the systemic immunity which follows one inoculation of dead vaccine might be sufficient to protect sheep against natural infection, and since the method appeared to be safe for large scale immunisation, it was decided that its value should be tested by field trials in 1931. Accordingly, during the winter of 1930-31 vaccines were prepared, by the method described earlier in this paper, from the brain and spinal cord of five sheep. These animals were infected by intracerebral inoculation with louping-ill virus, and were killed when showing typical symptoms of the disease. The details of production and testing are set out in the following table.

Sheep Number	28	39	982	995	1,000
Volume in c.c.	510	605	575	615	535
Potency test: virus detected at a concentration of	1 in 100	1 in 100	1 in 1,000	1 in 100,000	1 in 1,000,000
Sterility test for louping-ill virus by intracerebral inoculation of mice	0 0	0 0	0 0	0 0	0 0
Bacteriological sterility test	-	-	-	-	-

- = No bacterial contaminant detected.

0 = Mice remained healthy.

These five batches of vaccine were blended to make a uniform product, and the immunising value of the blended vaccine was tested in sheep. Three sheep (Nos. 1, 984 and 998) were inoculated subcutaneously with 5.0 c.c. of vaccine. Nine days later these animals, with three controls (Nos. 185, 186 and 187) were given a test dose of virus subcutaneously. The vaccinated animals were inoculated with 10 c.c. of a 1 in 100 saline suspension of dried brain 43, and the control animals received 10 c.c. of a 1 in 10,000 suspension of the same dry virus. The result of these tests is shown in fig. XVI, from which it will be seen that the vaccinated animals did not react to the test dose of virus, whereas the controls which received a hundred times less virus developed typical febrile reactions, and one of the three died of louping-ill.

Field Trial of Louping-ill Prophylactic Vaccine - 1931

This vaccine was subjected to field trial on eight farms in the spring of 1931. The experiment was confined to hoggs (yearling sheep) on certain farms where the disease was known to occur. Half of the hoggs were selected at random, and were vaccinated, whilst the other half were left untreated.

The/

The vaccine was inoculated subcutaneously inside the thigh, and the dose employed was 5.0 c.c. The vaccinated animals were reliably marked at the time of inoculation, so that they could be distinguished from the non-vaccinated animals throughout the louping-ill season. Thus, the vaccinated and non-vaccinated animals grazed together under the same conditions, and a record of the total death rate from all causes in each group was made. This method of recording the result was adopted, since it was believed that the majority of the deaths amongst the sheep on a louping-ill farm in the spring are primarily due to louping-ill infection, although many of the animals may die without showing diagnostic symptoms of the disease. In addition, vaccination would be of little value from a practical aspect, unless it were capable of reducing the gross mortality. In most seasons louping-ill occurs in its greatest incidence in the period between the middle of March and the middle of June. Accordingly, the sheep on the various farms included in the experiment were vaccinated during the period between the middle of March and the middle of April 1931. The farmers recorded the total death rate which occurred in the vaccinated and non-vaccinated animals, and returned/

returned the results at the end of July 1931. Fig. XVII presents the result diagrammatically, and the actual figures for each farm are also shown.

Thus, it will be seen that of 455 animals inoculated subcutaneously with 5.0 c.c. of "louping-ill prophylactic vaccine," 3 died: 0.66 per cent., whilst of 381 non-vaccinated animals grazing under the same conditions, 30 died: 7.87 per cent. There was evidence, therefore, that a formalinised vaccine, prepared from the tissues of the central nervous system obtained from sheep artificially infected with louping-ill was, within the limited scope of the figures available, capable of reducing the gross mortality which occurs amongst the sheep in the spring on a louping-ill farm. The figures were encouraging, but since the production of vaccine had been attempted only on an experimental scale, the confirmation to be obtained by a larger number of experiments was still required before definite conclusions could be drawn with regard to its value in reducing the mortality.

LOUPING-ILL PROPHYLACTIC VACCINE - 1932

During October, November and December 1931, and January and February 1932, vaccine was prepared from the brains and spinal cords, and in a number of cases from the spleens of 59 sheep infected with louping/

louping-ill by intracerebral inoculation with dry virus. The vaccine was prepared by the usual technique, in the same manner as that prepared in 1931. A summary of the tests carried out in the course of producing this vaccine is set out in the following table.

Abbreviations used in the Table

B & C = Brain and spinal cord.

+x = Death with an incubation period of x days.

+ op = Death due to operation.

The column headed "incubation period" contains a record of the incubation period following intracerebral inoculation of the sheep used for the production of vaccine.

Sheep Number	Incubation Period	Tissue Used	Volume in c.c.	Potency Test Concentration of Virus	Sterility Test for Virus	Sterility Test for Bacteria
191	7 days	B & C	516	1 in 10,000	0 0	-
215	7 days	B & C	516	1 in 10,000	+15 0	-
222	5 days	B & C	352	1 in 100	0 0	-
225	5 days	B & C	456	1 in 1,000	0 0	-
217	6 days	B & C	452	1 in 1,000	0 0	-
203	7 days	B & C	440	1 in 100,000	0 0	-
380	7 days	B & C	434	1 in 1,000,000	0 0	-
422	7 days	B & C	468	1 in 1,000,000	0 0	-
311	5 days	B & C	424	1 in 10,000	0 0	-
		Spleen	92	1 in 1,000	0 0	-
137	5 days	B & C	412	1 in 10,000	0 0	-
		Spleen	232	1 in 1,000	0 0	-
307	6 days	B & C	224	1 in 100,000	0 0	-
		Spleen	152	1 in 1,000	0 0	-
320	7 days	B & C	340	1 in 100,000	0 0	-
		Spleen	232	1 in 1,000	0 0	-
197	6 days	B & C	496	1 in 10,000	0 0	-
		Spleen	256	1 in 1,000	0 0	-
202	7 days	B & C	524	1 in 100,000	0 0	-
		Spleen	276	1 in 1,000	0 0	-
218	6 days	B & C	492	1 in 1,000,000	0 0	-
		Spleen	212	1 in 100	0 0	-
220	6 days	B & C	518	1 in 10,000	0 0	-
		Spleen	246	1 in 1,000	0 0	-
313	6 days	B & C	444	1 in 10,000	0 0	-
		Spleen	100	1 in 100	0 +15	-

Sheep Number	Incubation Period	Tissue Used	Volume in c.c.	Potency Test Concentration of Virus	Sterility Test for Virus	Sterility Test for Bacteria
304	6 days	B & C	376	1 in 10,000	+op 0	-
		Spleen	152	1 in 1,000	0 0	-
296	6 days	B & C	472	1 in 100,000	0 0	-
		Spleen	144	1 in 1,000	0 0	-
301	7 days	B & C	412	1 in 10,000	0 0	-
		Spleen	148	0	0 0	-
226	6 days	B & C	448	1 in 100,000	0 0	-
		Spleen	400	1 in 100,000	0 0	-
231	6 days	B & C	488	1 in 100,000	0 0	-
		Spleen	140	1 in 100	0 0	-
322	6 days	B & C	472	1 in 10,000	0 0	-
		Spleen	276	1 in 10,000	0 +4	-
315	6 days	B & C	388	1 in 100,000	0 0	-
		Spleen	220	1 in 100	0 0	-
295	6 days	B & C	428	1 in 100,000	0 0	-
		Spleen	208	1 in 100,000	0 0	-
331	6 days	B & C	472	1 in 10,000	+10 0	-
		Spleen	212	1 in 1,000	+24 0	-
366	5 days	B & C	448	1 in 10,000	0 0	-
		Spleen	172	1 in 1,000	+op 0	-
287	5 days	B & C	480	1 in 10,000	0 0	-
		Spleen	284	1 in 1,000	0 0	-
286	6 days	B & C	368	1 in 10,000	0 0	-
		Spleen	248	1 in 10,000	0 0	-
250	6 days	B & C	500	1 in 100,000	0 0	-
		Spleen	180	1 in 1,000	0 0	-

Sheep Number	Incubation Period	Tissue Used	Volume in c.c.	Potency Test Concentration of Virus	Sterility Test for Virus	Sterility Test for Bacteria
300	6 days	B & C	440	1 in 1,000	0 +16	-
		Spleen	220	1 in 1,000	0 0	-
245	6 days	B & C	438	1 in 100,000	0 0	-
		Spleen	144	0	0 0	-
282	5 days	B & C	480	1 in 1,000	0 0	-
		Spleen	172	1 in 1,000	+15 0	-
314	6 days	B & C	412	1 in 100,000	+3 0	-
		Spleen	164	1 in 10,000	0 0	-
317	6 days	B & C	352	1 in 100,000	0 0	-
		Spleen	88	1 in 100,000	0 0	-
321	6 days	B & C	448	1 in 1,000	+op 0	-
		Spleen	140	1 in 1,000	0 0	-
417	5 days	B & C	480	1 in 10,000	0 0	-
		Spleen	296	1 in 1,000	+op 0	-
419	10 days	B & C	464	1 in 1,000	0 0	-
		Spleen	240	0	0 0	-
411	11 days	B & C	472	0	0 0	-
		Spleen	212	0	0 0	-
414	5 days	B & C	468	1 in 1,000	0 0	-
		Spleen	160	1 in 1,000	0 0	-
365	6 days	B & C	412	1 in 1,000	0 0	-
		Spleen	168	1 in 10,000	0 0	-
361	6 days	B & C	436	1 in 1,000	0 0	-
		Spleen	116	1 in 10,000	0 0	-
379	7 days	B & C	460	1 in 100,000	0 0	-
		Spleen	224	1 in 100	0 0	-

Sheep Number	Incubation Period	Tissue Used	Volume in c.c.	Potency Test Concentration of Virus	Sterility Test for Virus	Sterility Test for Bacteria
418	7 days	B & C	384	1 in 1,000	0 +18	-
		Spleen	192	1 in 10,000	0 0	-
354	7 days	B & C	720	1 in 100,000	0 0	-
		Spleen	176	1 in 100	0 0	-
355	6 days	B & C	436	1 in 10,000	+16 +17	-
		Spleen	124	1 in 1,000	0 0	-
412	7 days	B & C	384	1 in 10,000	0 0	+
		Spleen	224	1 in 1,000	+4 0	-
998	8 days	B & C	532	1 in 100	+op +21	-
		Spleen	408	0	0 0	-
293	7 days	B & C		1 in 10,000	0 0	-
		Spleen		1 in 10,000	0 0	-
216	8 days	B & C	432	1 in 1,000	0 0	-
		Spleen	296	0	0 0	-
1	9 days	B & C	544	1 in 100	0 0	-
		Spleen	640	0	0 0	-
246	6 days	B & C	396	1 in 10,000	0 0	-
		Spleen	264	1 in 1,000	0 0	-
244	7 days	B & C	404	1 in 10,000	0 0	-
		Spleen	332	1 in 100	0 0	-
308	6 days	B & C	364	1 in 10,000	0 0	-
		Spleen	200	1 in 1,000	0 0	-
318	6 days	B & C	388	1 in 100,000	0 0	-
		Spleen	128	1 in 1,000	0 0	-
306	9 days	B & C	396	1 in 100,000	0 0	-
		Spleen	108	1 in 100	0 0	-
302	8 days	B & C	432	1 in 100,000	0 0	-
		Spleen	120	0	0 0	-
316	7 days	B & C	432	1 in 100,000	0 0	-
		Spleen	228	1 in 100	0 0	-
359	8 days	B & C	336	1 in 100,000	+op +19	-
		Spleen	192	1 in 1,000	0 0	-

Louping-ill Prophylactic Brain and Cord Vaccine - 1932

The vaccine prepared from the brains and spinal cords of all the sheep noted in the foregoing summary (with the exception of those noted in red) were blended to make a uniform product. The batches of vaccine which were excluded from the final mixing were discarded either because there was a doubt about there being a sufficient concentration of virus in the tissue; because there was a definite bacterial contaminant; or because the mice inoculated intracerebrally with the product died from an obscure cause. The blended vaccine was designated "louping-ill prophylactic brain and cord vaccine - 1932."

Testing of Louping-ill Prophylactic Brain and Cord Vaccine - 1932

The louping-ill prophylactic brain and cord vaccine - 1932 was tested in the following manner:-

(a) Sterility Test in Mice. Four mice were inoculated intracerebrally with 0.05 c.c. of vaccine, and four with the vaccine diluted to 1 in 10 with saline. All the mice remained healthy whilst under observation for 21 days.

(b)/

(b) Bacteriological Sterility Test. Several 250 c.c. bottles of horse flesh broth and several slopes of blood agar were inoculated with 0.5 c.c. of vaccine and incubated for seven days. No bacterial contaminant was detected.

(c) Test in Sheep for Antigenic Value. The immunising value of the vaccine was determined by subcutaneous inoculation of five sheep with a dose of 5.0 c.c. These animals did not develop any reaction after vaccination, and when tested along with suitable controls by subcutaneous inoculation with living virus, the vaccinated animals did not react, whereas the control animals developed a febrile reaction and definite constitutional disturbance. The result of this test is shown in fig. XVIII.

Louping-ill Prophylactic Spleen Vaccine - 1932

The vaccine prepared from the spleens of sheep noted in the summary of the production (with the exception of those noted in red) were blended to make/

make a uniform product which was designated "louping-ill prophylactic spleen vaccine"- 1932.

Testing of Louping-ill Prophylactic Spleen Vaccine - 1932

The prophylactic spleen vaccine was tested by the same methods as those employed in testing the prophylactic brain and cord vaccine. The vaccine was bacteriologically sterile and did not produce disease when inoculated intracerebrally into mice. When tested in sheep for immunising value, it proved to be a good antigen. The result of this test is shown in fig. XIX.

Field Trial of Louping-ill Prophylactic Vaccine - 1932 for the Prevention of Louping-ill in Hoggs

The brain and cord vaccine and the spleen vaccine prepared in the winter of 1931-32 were blended to make a uniform product, and this was subjected to a field trial similar to that of 1931. The experiment was carried out on 23 farms on which half of the number of yearling sheep were vaccinated and half left untreated. Fig. XX presents the result diagrammatically, and shows the figures for every farm. Thus, of 1172 animals which were vaccinated, 10 died: 0.85 per cent., whilst of 1208 non-vaccinated animals grazing under the same conditions/

conditions, 88 died: 7.28 per cent. This result confirmed the preliminary experiments carried out in 1931, and showed that the vaccine was capable of effecting a considerable reduction in the gross mortality amongst the yearling sheep on farms where louping-ill was prevalent.

Field Trial of Louping-ill Prophylactic Vaccine - 1932 for the Prevention of Louping-ill in Lambs

It is generally recognised that the greatest mortality from louping-ill occurs in lambs, and since the prophylactic vaccine was apparently safe for use in hoggs, it was decided to test its value on one farm for the prevention of the disease in lambs. The dose of vaccine employed was 3.0 c.c. inoculated subcutaneously inside the thigh. By arrangement, the vaccine was supplied to a farmer who, after instruction in the method of vaccination, carried out the experiment. The following extract taken from the owner's letter, dated May 30th, 1932, describes the nature of the experiment:-

> "On the 11th and 12th of May I drew into the sheep folds Blackface ewes and lambs, and inoculated 104 lambs, whose ages ranged from 2 days to 4 weeks. The lambs suffered no ill effect and mothered up well afterwards. At this date there were about 350 lambs on the hill and I had lost about 14 from louping-ill. The weather was, and since has been, bad louping-ill weather. Between the 11th May and 28th May I lost 30 control lambs; 16 of these were considered/

considered to have died from louping-ill and 14 from other causes, although some of these exhibited symptoms of louping-ill. Amongst the inoculated lambs there has been one death due to grass-ill.

What has struck me most in this trial is the large number of lambs which have died in the controls - 12 per cent. against a total of 1 per cent. inoculated. Also, while in the controls there are several lambs hanging legs, etc., there is none of this in the inoculated lambs."

The final result received in August stated that out of 104 lambs vaccinated, 3 died, whilst out of 246 controls, 39 died. The 3 inoculated lambs which died were not considered to have died of louping-ill. Of the 39 control lambs which died, 16 were believed to be due to louping-ill, and 23 to other causes. In all probability, a number of the latter were atypical cases of louping-ill. Fig. XXI depicts the result.

LOUPING-ILL PROPHYLACTIC VACCINE - 1933

Field Trial of Louping-ill Prophylactic Vaccine - 1933 for the Prevention of Louping-ill in Hoggs

During the winter 1932-33 louping-ill vaccine was prepared in bulk so that an extensive field trial could be carried out in the spring of 1933. This vaccine was prepared in the usual way from selected brains, spinal cords and spleens of sheep infected with louping-ill by intracerebral inoculation with virus./

virus. The vaccine was shown to be bacteriologically sterile; it did not cause louping-ill when inoculated intracerebrally into mice or sheep; and it was capable of protecting sheep against a dose of living virus inoculated subcutaneously. The field trials were designed firstly to continue the testing of the prophylactic value of the vaccine in hoggs, and secondly, to make an extended trial of the vaccine for the prevention of louping-ill in lambs. As in the previous experiments, half the number of animals which grazed together on the same pasture were vaccinated, and half left untreated. Hoggs were given a dose of 5.0 c.c. subcutaneously, and lambs a dose of 3.0 c.c. by the same route. Fifteen thousand doses were issued for the inoculation of lambs and 7,000 doses for the inoculation of hoggs. Forms for recording the result were sent to farmers, who returned a record of the number of animals in each group which died. They were also asked to state what, in their opinion, was the cause of death. When the results were collected, it was found that on many farms where a considerable death rate had been expected, no animals died, either in the vaccinated or non-vaccinated groups. The records for these farms have been excluded from the final figures, but the results on all farms on which a mortality occurred, either/

either in the vaccinated or non-vaccinated animals, have been included. Thus:-

of 5,084 vaccinated hoggs
124 died from all causes 2.43%

of these deaths,
50 were attributed to louping-ill 0.98%

Of 5,429 non-vaccinated hoggs,
354 died from all causes 6.70%

of these deaths,
237 were attributed to louping-ill 4.49%

The total death rate was, therefore, reduced from 6.70 per cent. to 2.49 per cent., and the death rate attributed to louping-ill was reduced from 4.49% to 0.98 per cent. The result obtained on each of 105 farms is set out in fig. XXII.

It will be seen that on fifteen of these farms, the percentage gross mortality was greater in the vaccinated than in the non-vaccinated animals. Closer examination of these results, however, shows that they are of little significance in the mass result; for example, on Farm No. 70 there were only 7 vaccinated animals, of which 1 died:14.3 per cent., whilst out of 13 non-vaccinated animals, 1 died: 7.7 per cent. In forming an opinion on the result, it has to be remembered firstly that the vaccine was applied by the farmers, and it is probable that

imperfect/

imperfect vaccination of some animals occurred and, secondly, the diagnosis accepted was the one given by the farmer. On most of the other farms there was a definite reduction in the mortality from all causes in the vaccinated animals as compared with that in the non-vaccinated animals.

Field Trial of Louping-ill Prophylactic Vaccine - 1933 for the Prevention of Louping-ill in Lambs.

The result obtained in the field trial of the vaccine for the prevention of louping-ill in lambs has been prepared in the same manner as the result in hoggs. Again, the results indicate that the death rate can be reduced by vaccination. Thus:-

of 9,032 vaccinated lambs, 412 died from all causes	4.47%
of these deaths, 128 were attributed to louping-ill	1.40%
Of 10,137 non-vaccinated lambs, 860 died from all causes	8.48%
of these deaths, 447 were attributed to louping-ill	4.40%

The total death rate was, therefore, reduced from 8.48 per cent. to 4.47 per cent., and the death rate attributed to louping-ill was reduced from 4.40 per cent. to 1.40 per cent. The incidence of louping-ill during the 1933 season is generally regarded as having/

having been exceptionally low, and in consequence it might be suggested that the protective value of the vaccine was not subjected to a severe test. The result obtained on the various farms is set out in fig. XXIII, from which it will be seen that although the average death rate was low, there are certain farms on which a heavy death rate did occur. For example, on Farm No. 87, there were 57 lambs vaccinated, and none of these died, whereas, out of 63 non-vaccinated lambs, 24 died from all causes, and 21 of these were attributed to louping-ill - a 38.1 per cent. mortality, as compared with nil. This result suggests that, ~~even~~ in a season when the incidence is high, vaccination will be of definite value for the control of the disease.

DISCUSSION

In addition to the experimental evidence presented in this paper regarding the value of a formalinised vaccine for the prevention of louping-ill in sheep, it has to be remembered that the field inoculations were carried out in the spring, and the results collected in July and August of the same year; consequently, the total death rate for the whole year, and also the death rate in subsequent years, have not been taken into consideration. Unfortunately, accurate figures for these results are not available, but information has been received from many farmers that a number of sheep died in the autumn of 1933 after the field results had been collected, and the mortality was apparently confined to the non-vaccinated animals. In the case of the hoggs which were vaccinated in 1931, there has been a very low mortality in these animals during 1932 and 1933, whereas a considerable number of deaths has occurred in the non-vaccinated animals on the same farms. In all probability this immunity is not due to vaccination alone, but to vaccination assisted by subsequent natural infection. At the present time a single inoculation only has been employed, and, if necessary, the degree of immunity could, in all probability, be increased/

increased by a double inoculation. This season the latter method is being adopted on many farms where hoggs, which were vaccinated as lambs in 1933, have been inoculated for a second time in March 1934. This method of applying the vaccine will probably be the one eventually adopted for routine use, since by vaccination of the lambs the death rate in their first year of life would be considerably reduced, and if the same animals were again vaccinated as hoggs, they would probably have sufficient immunity to withstand infection until they were six years old, at which age the hill ewes are generally sold.

In the ordinary course of sheep husbandry on hill farms, two classes of sheep should be available for sale each year; firstly, the surplus lambs not required to keep up the stock and, secondly, the old ewes. On farms where louping-ill is prevalent, it is rare, if ever, that any ewe lambs are available for sale - all require to be kept for stock - and the number of old ewes for sale is generally much below the production capacity of the farm owing to the toll of deaths exacted by louping-ill over a period of years. On farms where vaccination was commenced in 1931, there has been a marked increase in the number of lambs available for sale, because the number required to keep up the stock can ~~not~~ be reduced owing to the decrease in the death rate. It

is/

is also anticipated that the number of old ewes available for sale on these farms will increase, and the preliminary information obtained on this point indicates that the increase will be considerable.

Since this investigation has reached a stage at which the sheep owners are playing an important part in the field observations in progress, it is perhaps appropriate to quote the following abstract from a letter received from a sheep farmer, who has vaccinated one-half of his hoggs each season since 1931, and, in addition, one-half of the lambs were vaccinated in 1933. The letter dated 8th February, 1934, was received when arrangements were being made for field trials this season.

> "As regards the ewe hoggs - I have a total of 710 hoggs away at winterings. Allowing for casualties, I expect to have roughly 700 to bring home. This number is, however, more than I require for stock as, thanks to our braxy and louping-ill inoculations, the sheep are now living and not dying as they did before. I propose, therefore, to draw out 100 of the hoggs (the worst of them) and sell these at the June sales. I will not bring these hoggs home, but leave them away and take them straight off their grazing grounds to the sales.
>
> I may mention that we have never before been in a position to sell any hoggs, and the head shepherd says that in all the 25 years he has been here he has never known ewe hoggs to be sold before off these farms.
>
> Personally, I am so convinced of the efficacy of inoculating against louping-ill that I would like to inoculate the whole 600 hoggs we are keeping for stock, and am prepared to/

to pay for the vaccine, but if you still wish me to do half of my ewe hoggs so as to carry on your experiment, I will agree to do so, although I would much prefer to inoculate the whole lot."

This farm is of particular interest since the owner has kept a very accurate account of his sheep over a period of years. Prior to 1932, the number of ewe lambs kept for stock was generally 800, and by the time six years had elapsed, only about 250 of these remained alive to be sold as old ewes. This appalling death rate was due mainly to louping-ill, and, in a lesser degree, to an anaerobic infection of sheep known as "braxy."

Finally, when prophylactic vaccination of all the animals on infected farms has been carried out for a number of years, it is probable that very few, if any, of the ticks on the pasture will be harbouring the virus of louping-ill, unless there exists on hill farms an alternative host for ticks which is susceptible to louping-ill.

CONCLUSION

The mortality due to louping-ill, an encephalomyelitis of sheep, can be controlled by means of suitable vaccines, which consist of crude louping-ill virus inactivated by formaldehyde.

ACKNOWLEDGMENTS.

The work recorded in this thesis was carried out under the aegis of the Animal Diseases Research Association, and I wish to record my sense of gratitude to those who have taken an interest in the investigation and encouraged the work.

To the Scientific Committee of the Association under the convenorship of Professor T. J. Mackie.

To the Director of this Institute Dr. J. Russell Greig for the way in which he has fostered the work.

To the many agriculturists who placed their sheep at my disposal for field experiments.

I am greatly indebted to my colleagues Messrs. A. Brownlee and D.R. Wilson, and Dr. J. MacLeod, for the very loyal way in which they have supported me during the investigation, and for their very valuable work.

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Figure I

The arrows on the underside of the curve indicate times of withdrawal of blood for the intracerebral inoculation of mice.

M = Mouse inoculated intracerebrally.

O = No infection.

+x = Death from louping-ill with an incubation period of x days.

Ic = Inoculated intracerebrally.

Taken from
J. Comp. Path. & Ther., XLV, 106, June 1932.

"Studies in Louping-ill. I"

by

W. S. Gordon,
A. Brownlee,
D. R. Wilson
and
J. MacLeod.

Figure I

TEMPERATURE CHART OF A SHEEP AFTER INTRACEREBRAL INOCULATION WITH THE VIRUS OF LOUPING-ILL.

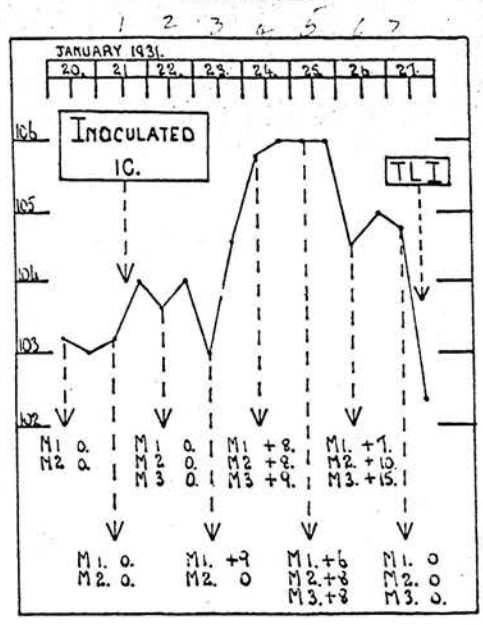


Figure II

The arrows on the underside of the curve indicate times at which blood was withdrawn for intracerebral inoculation of mice.

+x = Death from louping-ill with an incubation period of x days.

0 = No infection.

FIGURE II.

Temperature Chart of a Sheep after Subcutaneous Inoculation with the Virus of Louping-ill.

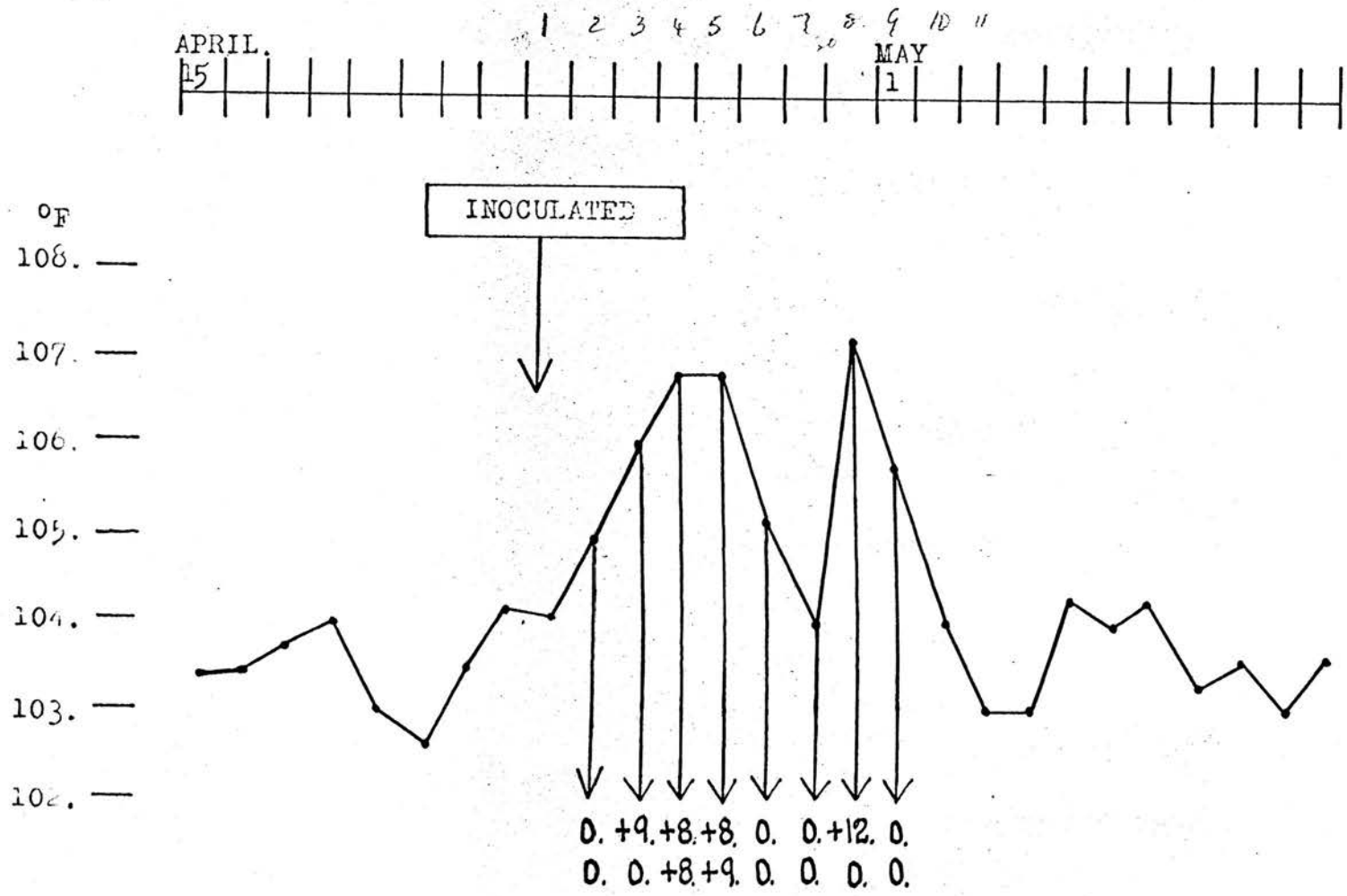


Figure III

Taken from
J. Comp. Path. & Ther., XLV, 106, June 1932

"Studies in Louping-ill. I"

by

W. S. Gordon,
A. Brownlee,
D. R. Wilson
and
J. MacLeod.

Figure III

COMPOSITE TEMPERATURE CHART OF "IMMUNES" AND "CONTROLS" DURING THE PERIOD OF EXPOSURE TO LOUPING-ILL INFECTION ON A "DISEASED FARM."

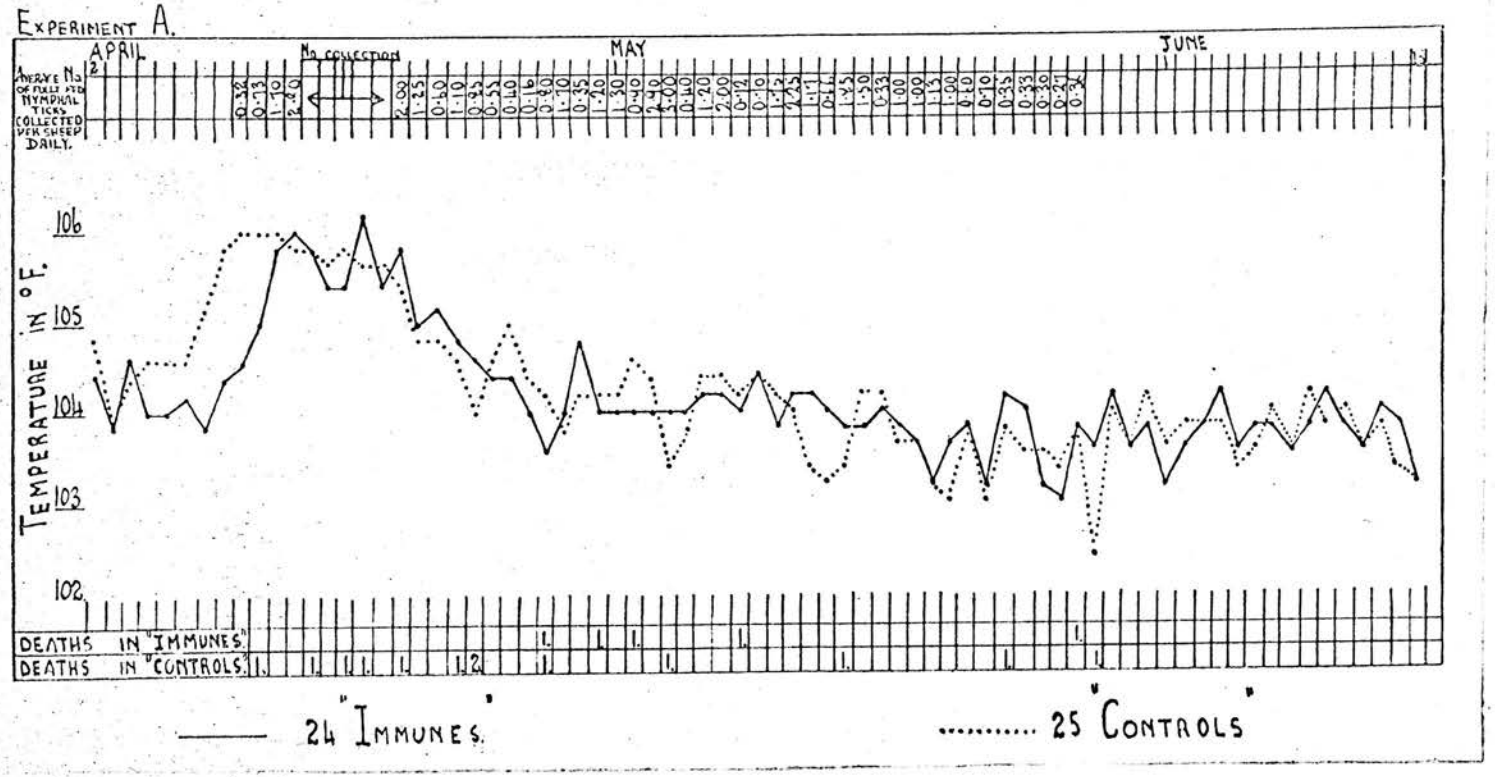


Figure IV

ICR = Inoculated intracerebrally with louping-ill virus.

\+ = Death due to louping-ill.

0 = No infection.

T.L.I. = Typical louping-ill.

The three stages in the life cycle of the tick require different periods of time for engorgement.

Larvae require about three to four days.
Nymphs require about five to six days.
Females require about eight to nine days.

To ensure that each stage has an opportunity of acquiring virus, the host sheep is infested with the different stages, as shown in the diagram.

Taken from
Veterinary Record, XIV, 1, January 1934.

"The Control of Certain Diseases of Sheep"

by

W. S. Gordon.

Figure IV

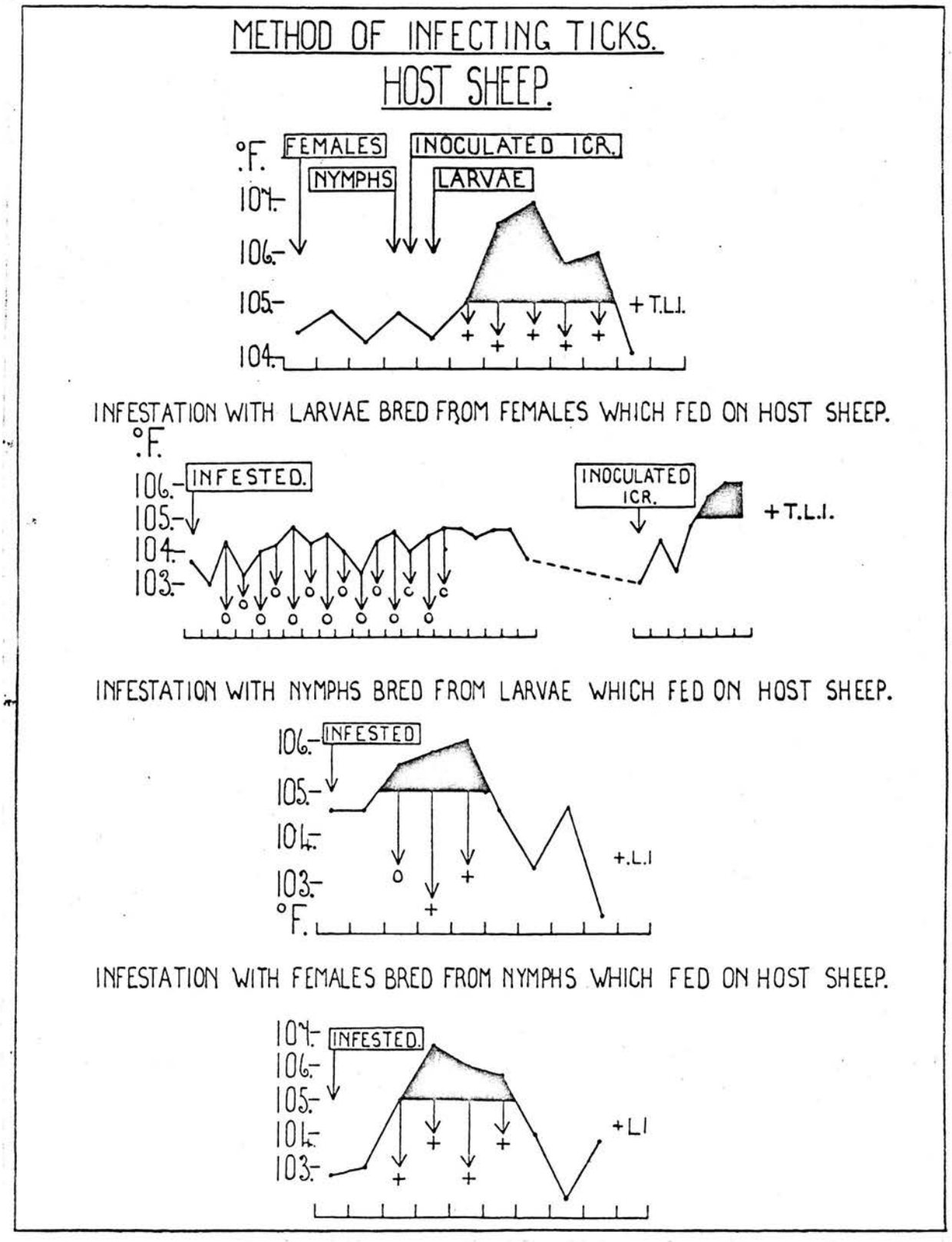

METHOD OF INFECTING TICKS.
HOST SHEEP.
°F.
FEMALES
INOCULATED ICR.
NYMPHS
LARVAE
107.
106.
105.
104.
+ T.L.I.
INFESTATION WITH LARVAE BRED FROM FEMALES WHICH FED ON HOST SHEEP.
°F.
106.
105.
104.
103.
INFESTED.
INOCULATED ICR.
+ T.L.I.
INFESTATION WITH NYMPHS BRED FROM LARVAE WHICH FED ON HOST SHEEP.
106.
INFESTED
105.
104.
103.
°F.
+.L.I
INFESTATION WITH FEMALES BRED FROM NYMPHS WHICH FED ON HOST SHEEP.
107.
INFESTED.
106.
105.
104.
103.
+L.I

Figure V

+	=	Death from louping-ill.
±	=	Death from undetermined cause within a louping-ill incubation period.
0	=	No infection.
+op	=	Death due to operation.
C.N.S.	=	Central nervous system.
T.L.I.	=	Typical louping-ill.

Sheep transferred to diseased farm - April 12th.
Sheep returned from diseased farm - June 3rd.

Tick-borne fever immunity test - June 13th.
Louping-ill immunity test - July 14th.

On post mortem examination of certain sheep, e.g. No. 504, the cause of death was attributed to pleurisy and pneumonia. This animal showed no symptoms of louping-ill before death, and louping-ill infection would not have been suspected if the specific virus had not been detected in the blood during a natural febrile reaction.

Sheep No..

Arrows on underside of curves indicate days on which blood was drawn and inoculated into mice.

TREATED 5 TO 7 STAINED PAR[illegible]

RETURNED AND DISEASED PART.

501. 108. °F. 105. 102.

502. 108. 105. 102. FOUND DEAD.

503. 108. 105. 102. DESTROYED.

504. 108. 105. 102. DESTRO[illegible]

505. 108. 105. 102.

506. 108. 105. 102. DESTROYED.

507. 108. 105. 102. FOUND DEAD.

508. 108. 105. 102.

509. 108. 105. 102.

510. 108. 105. 102.

511. 108. 105. 102. FOUND DEAD.

512. 108. 105. 102. DESTROYED.

513. 108. 105. 102. FOUND [illegible]

514. 105. 102.

515. 108. 105. 102.

516. 108. 105. 102. FOUND DEAD.

517. 108. 105. 102. DESTROYED.

518. 108 105 102 FOUND DEAD.

519. 108 105 102

520. 108 105 FOUND DEAD.

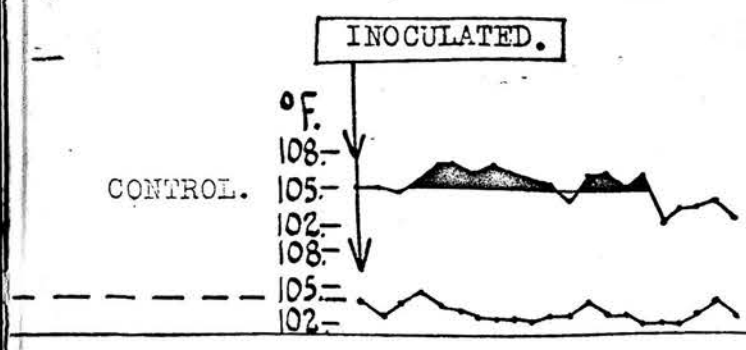
FIGURE V.

CASES OF 20 CONTROLS (i.e. 3 DEP[illegible] TO [illegible] SUSCEPTIBILITY TO LOUPING-ILL AND TICK-BORNE FEVER) WITH [illegible]

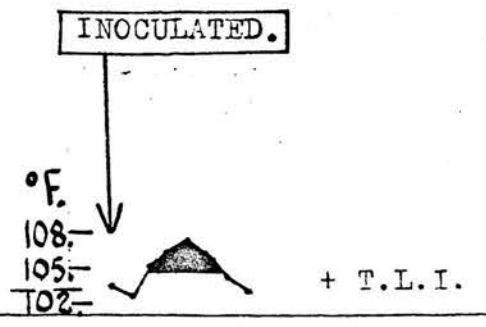
Result of post mortem.	Result of mouse inoculations for detection of louping-ill virus in tissues.			Summary of histological examination.
Terminal congestion of one lung, slight congestion of kidneys.	C. N. S.	±19.	0.	Cerebellum, medulla, cervical and lumbar cord. No lesions.
	Spleen.	±5.	0.	
	Pancreas.	0.	0.	
Pneumonia and abscess formation in the ventral parts of the lungs. Well marked pleuritic adhesions on one side.	C. N. S.	±20.	0.	Cerebellum, medulla, cervical and lumbar cord. No lesions.
	Spleen.	0.	0.	
	Pancreas.	+op.	0.	
Fibrinous pleurisy and pneumonia affecting left lung.	C. N. S.	±13.	0.	Cerebrum, cerebellum, medulla, cervical, thoracic and lumbar cord. No lesions.
	Spleen.	±11.	0.	
	Pancreas.	±5.	0.	
Pleurisy and pneumonia.	C. N. S.	±5.	0.	Cerebellum, medulla, cervical and lumbar cord. No lesions.
	Spleen.	±12.	0.	
	Pancreas.	0.	0.	
Pneumonia	C.N.S.	±5.	±17.	Cerebellum. No lesions. Medulla. A few inflammatory cells round an occasional vessel in the substance. Cervical and lumbar cord. No lesions.
	Spleen.	0.	0.	
	Pancreas.	0.	0.	
Pneumonia.	C.N.S.	+8.	+8.	Cerebellum and medulla. No lesions found. Cervical cord. Very slight infiltration of round cells into meningeal septa. Lumbar cord. No lesions found. Liver, spleen, kidney and pancreas. No lesions found.
	Spleen.	+5.	+14.	
	Pancreas.	+9.	+11.	
Pneumonia affecting lower parts of lungs.	C.N.S.	0.	0.	Cerebellum, medulla, cervical and lumbar cords. No lesions found.
	Spleen.	0.	0.	
	Pancreas.	±14.	0.	Cerebrum. Fairly well marked cellular infiltration into meninges and into perivascular sheaths of substance. (Many of these cells polymorphs.) Cellebrum. A few round cells in the meninges and perivascular sheaths of vessels of substance. Medulla. Fairly well marked perivascular infiltration of many of the vessels of substance. Cervical, dorsal and lumbar cords. No lesions found.
Pneumonia. Excess of peritoneal and pericardial fluid.	C.N.S.	0.	0.	
	Spleen.	0.	0.	
	Pancreas.	0.	0.	
Rupture of liver haemorrhage into peritoneal cavity. Organs anaemic.	C.N.S.	±14.	0.	Cerebellum, medulla, cervical and lumbar cords. No lesions found.
	Spleen.	0.	0.	
	Pancreas.	0.	0.	
Lower borders of lungs solid and containing small abscesses. Pleurisy.	C.N.S.	±10.	0.	Cerebellum, medulla, cervical and lumbar cords. No lesions found.
	Spleen.	0.	0.	
	Pancreas.	±17.	0.	
Areas of peritonitis on abomasum and rumen. ADVANCED POST-MORTEM CHANGES.	C.N.S.	+op.	+op.	Cerebellum. A few round cells in meninges and in perivascular sheaths of vessels of substance. Slight decrease in number of Purkinje cells. Medulla. Slight perivascular infiltration round occasional vessel in substance. Occasional nerve cells necrotic and surrounded by a few microglial cells. Occasional focus of microglia unrelated to any structure. Cervical cord. Slight round cell infiltration into depths of meningeal fold. Lumbar cord. Slight perivascular infiltration round occasional vessel.
	Spleen.	+op.	+op.	
	Pancreas.	+op.	+op.	
Pneumonia affecting lower parts of lungs. Pleurisy.	C.N.S.	+8.	+8.	Cerebellum and medulla. Slight cellular infiltration into meninges and into perivascular sheaths of vessels of substance. Cervical and lumbar cords. No lesions found. Pancreas, liver spleen, and kidney. No lesions found.
	Spleen.	+6.	+7.	
	Pancreas.	+7.	0.	

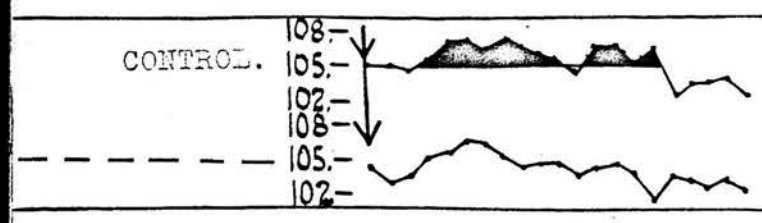
ON THE TICK INFESTED FARM.

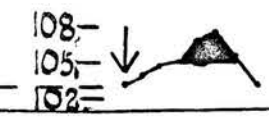
Tick-borne fever immunity test.
Subcutaneous inoculation with virulent blood.

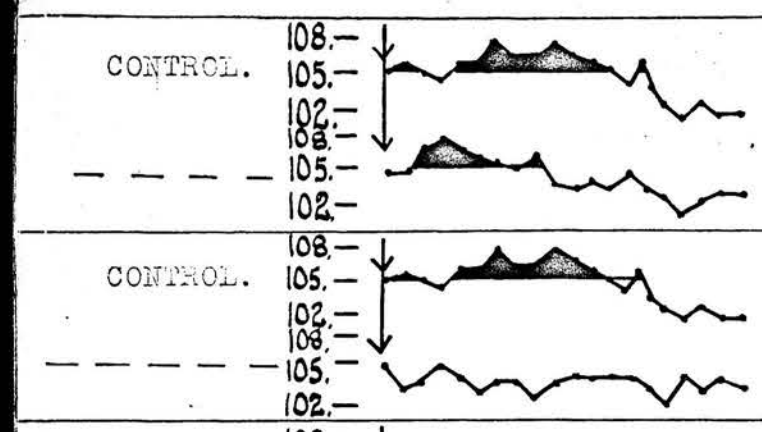


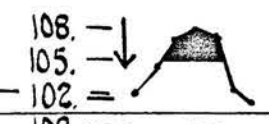
Louping-ill immunity test.
Intracerebral inoculation with louping-ill virus.

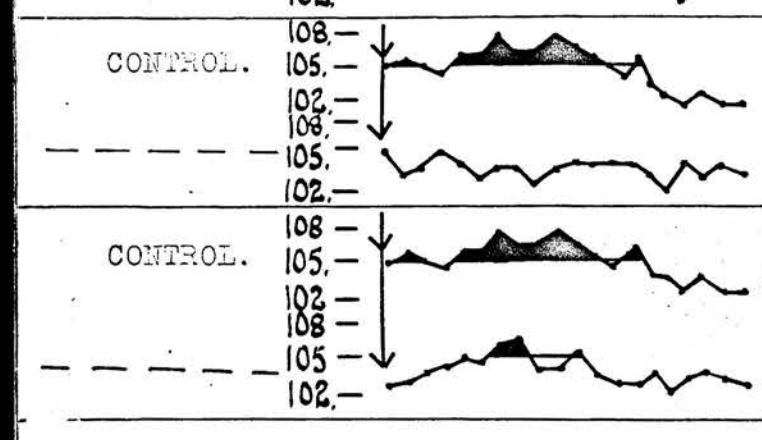


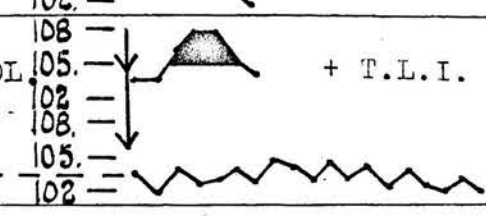


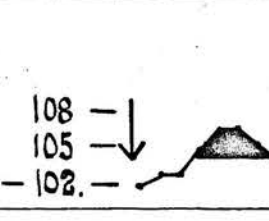


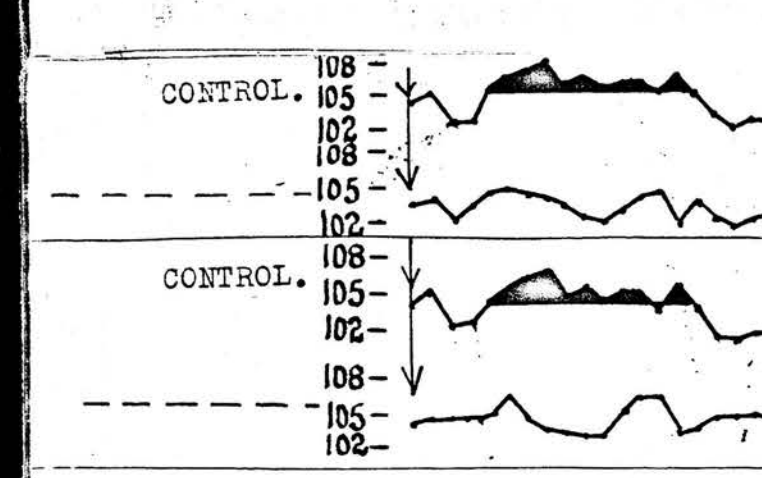


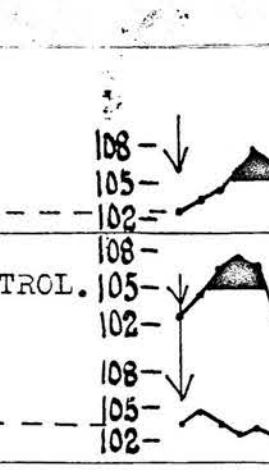


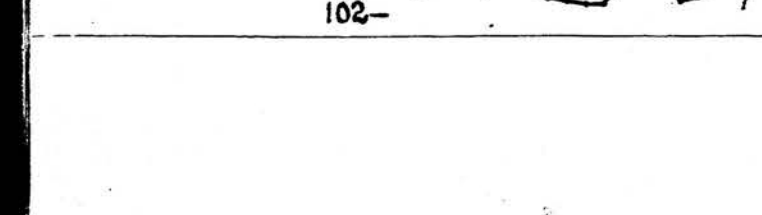


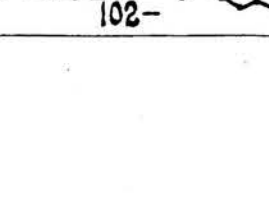


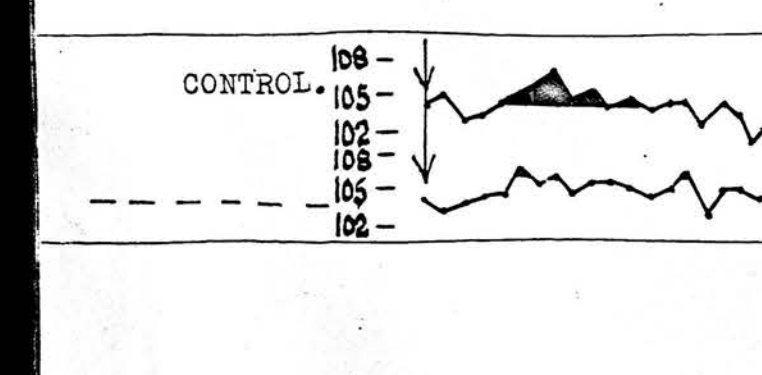












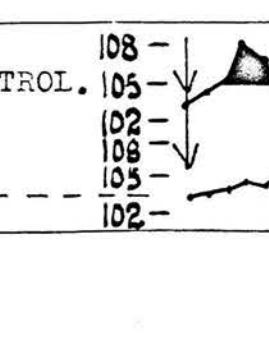


FIGURE VI

Comparative count of the nymphal ticks on the head and ears of sheep within the enclosure with that of the sheep outside the enclosure.

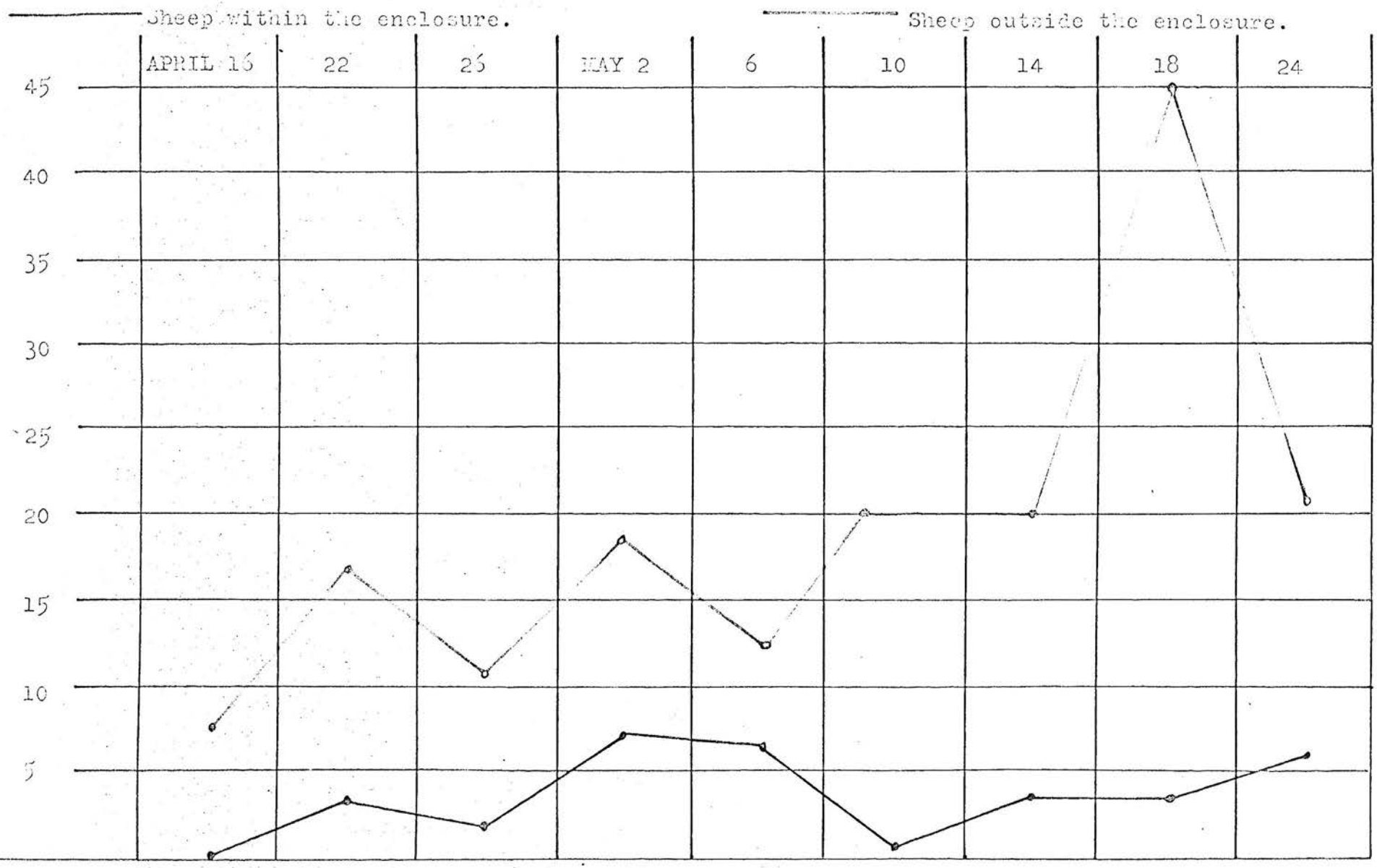


Figure VII

+ T.L.I. = Death due to typical louping-ill.

Sheep transferred to diseased farm - April 12th.
Sheep returned from diseased farm - June 3rd.

Tick-borne fever immunity test - June 13th.
Louping-ill immunity test - July 14th.

FIG. 2

TEMPERATURE CHARTS OF 10 GUINEA PIGS INFECTED WITH
<u>FEVER</u> WHILE CONFINED ON A RAISED ENCLOSURE OF THE PLAIN.

(PROOF ON PARTIALLY TICK PAPER.)

Arrows on underside of curves indicate days on which blood was drawn and inoculated into mice.

<u>Pig - non fever immunity test.</u>
Subcutaneous inoculation with virulent blood.

<u>Louping-ill immunity test.</u>
Intracerebral inoculation with louping-ill virus.

MAY | JUNE | JULY

CONFINED TO DISTANCE PARK. | RETURNED FROM DISTANCE PARK.

INOCULATED.

°F. 108. 105. 102.

CONTROL.

+ L.I.

483.

488.

489.

490.

Figure VIII

\+ = Death from louping-ill.

± = Death from undetermined cause within a louping-ill incubation period.

0 = No infection.

+op = Death due to operation.

Sheep transferred to diseased farm - April 12th.
Sheep returned from diseased farm - June 3rd.

Tick-borne fever immunity test - June 13th.
Louping-ill immunity test - July 14th.

FIGURE XIII.

TEMPERATURE RECORDS OF 10 SHEEP IMMUNE TO TICK-BORNE FEVER WHILE GRAZED ON THE TICK INFESTED FARM.

Arrows on underside of curves indicate days on which blood was drawn and inoculated into mice.

Tick-borne fever immunity test. — Subcutaneous inoculation with virulent blood.

Louping-ill immunity test. — Intracerebral inoculation with louping-ill virus.

Result of post mortem.	Result of mouse inoculations for detection of louping-ill virus in tissues.		Summary of histological examination
No definite lesions found.	C.N.S.	0. 0.	Cerebellum, medulla and cervical cord. Slight perivascular infiltration round a few of the vessels of the substance. Lumbar cord. No lesions. Pancreas, liver, kidney and spleen. No lesions.
	Spleen	0. 0.	
	Pancreas.	0. 0.	

Result of Post mortem.	Result of mouse inoculations for detection of louping-ill virus in tissues.		Summary of histological examination.
Acute diffuse inflammation of abomasum.	C.N.S.	±13. 0.	Cerebellum. A few cells round a few vessels of the substance. Medulla and cervical cord. No lesions. Lumbar cord. Slight cellular infiltration into meningeal septa.
	Spleen.	+op +op.	
	Pancreas.	+op. ±13	

Figure IX

\+ = Death from louping-ill.

± = Death from undetermined cause within a louping-ill incubation period.

0 = No infection.

+op = Death due to operation.

Sheep transferred to diseased farm - April 12th.
Sheep returned from diseased farm - June 3rd.

Tick-borne fever immunity test - June 13th.
Louping-ill immunity test - July 14th.

FIGURE IX.

TEMPERATURE RECORDS OF 10 SHEEP IMMUNE TO LOUPING-ILL, WHILE GRAZED ON THE TICK INFESTED FARM.

Arrows on underside of curves indicate days on which blood was drawn and inoculated into mice.

Tick-borne fever immunity test. Subcutaneous inoculation with virulent blood.

Louping-ill immunity test. Intracerebral inoculation with louping-ill virus.

SHEEP NO. | °F | TRANSFERRED TO DISEASED FARM. | RETURNED FROM DISEASED FARM. | INOCULATED. | INOCULATED.

464. °F 108 105 102 — CONTROL. °F 108 105 102 — °F 108 105 102 — + T.L.I.

465. 108 105 102 — CONTROL. 108 105 102 — 108 105 102 — + T.L.I.

47H. 108 105 102 — CONTROL. 108 105 102 — CONTROL. 108 105 102 — + T.L.I.

485 108 105 102 — CONTROL. 108 105 102 — 108 105 102 — + T.L.I.

487 108 105 102 — CONTROL. 108 105 102 — CONTROL. 108 105 102 — + T.L.I. SURVIVED

466 108 105 102 — CONTROL. 108 105 102 — CONTROL. 108 105 102 — + T.L.I. SURVIVED.

472 108 105 102 — CONTROL. 108 105 102 — CONTROL. 108 105 102 — + T.L.I. SURVIVED.

475 108 105 102 — ± DESTROYED.

Result of post mortem.

Pneumonia affecting the lower part of the lungs. Areas of pleuritic adhesions.

Result of mouse inoculations for detection of louping-ill virus in tissues.

C.N.S. 0. 0.

Spleen. 0. 0.

Pancreas. 0. 0.

Summary of histological examination.

Cerebellum, medulla, cervical and lumbar cord. No lesions.

480. 108 105 102 — ± ± CONTROL. 108 105 102 — 108 105 102 — + T.L.I.

482 108 105 102 — CONTROL. 108 105 102 — 108 105 102 — + T.L.I.

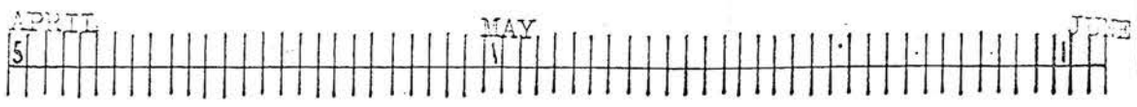
Figure X

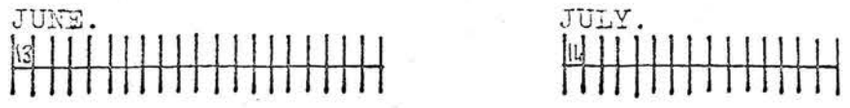
+ T.L.I. = Death due to typical louping-ill.

Sheep transferred to diseased farm - April 12th.
Sheep returned from diseased farm - June 3rd.

Tick-borne fever immunity test - June 13th.
Louping-ill immunity test - July 14th.

TEMPERATURE RECORDS OF 10 SHEEP IMMUNE TO LOUPING-ILL AND TICK-BORNE FEVER WHILE GRAZED ON THE TICK-INFESTED FARM.





Arrows on underside of curves indicate days on which blood was drawn and inoculated into mice.

Tick-borne fever immunity test.

Subcutaneous inoculation with virulent blood.

Louping-ill immunity test.

Intracerebral inoculation with louping-ill virus.

Figure XI

Immunity of the central nervous system following subcutaneous inoculation with living virus.

FIGURE XI

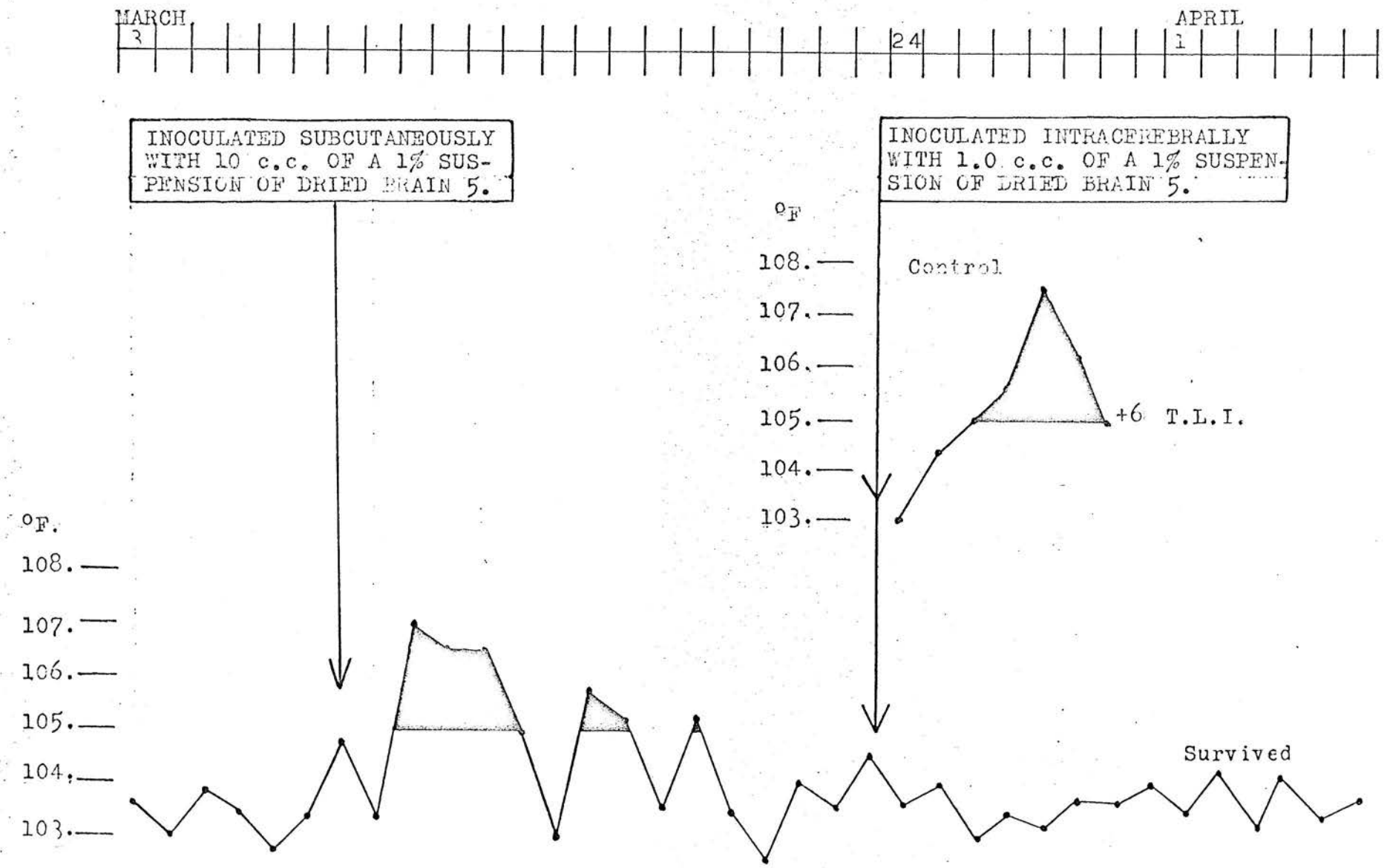

MARCH
3
24
APRIL
1
INOCULATED SUBCUTANEOUSLY WITH 10 c.c. OF A 1% SUSPENSION OF DRIED BRAIN 5.
INOCULATED INTRACEREBRALLY WITH 1.0 c.c. OF A 1% SUSPENSION OF DRIED BRAIN 5.
°F
108.
107.
106.
105.
104.
103.
Control
+6 T.L.I.
°F.
108.
107.
106.
105.
104.
103.
Survived

Figure XII

Central nervous system not immune after one inoculation of dead vaccine subcutaneously.

FIGURE XII

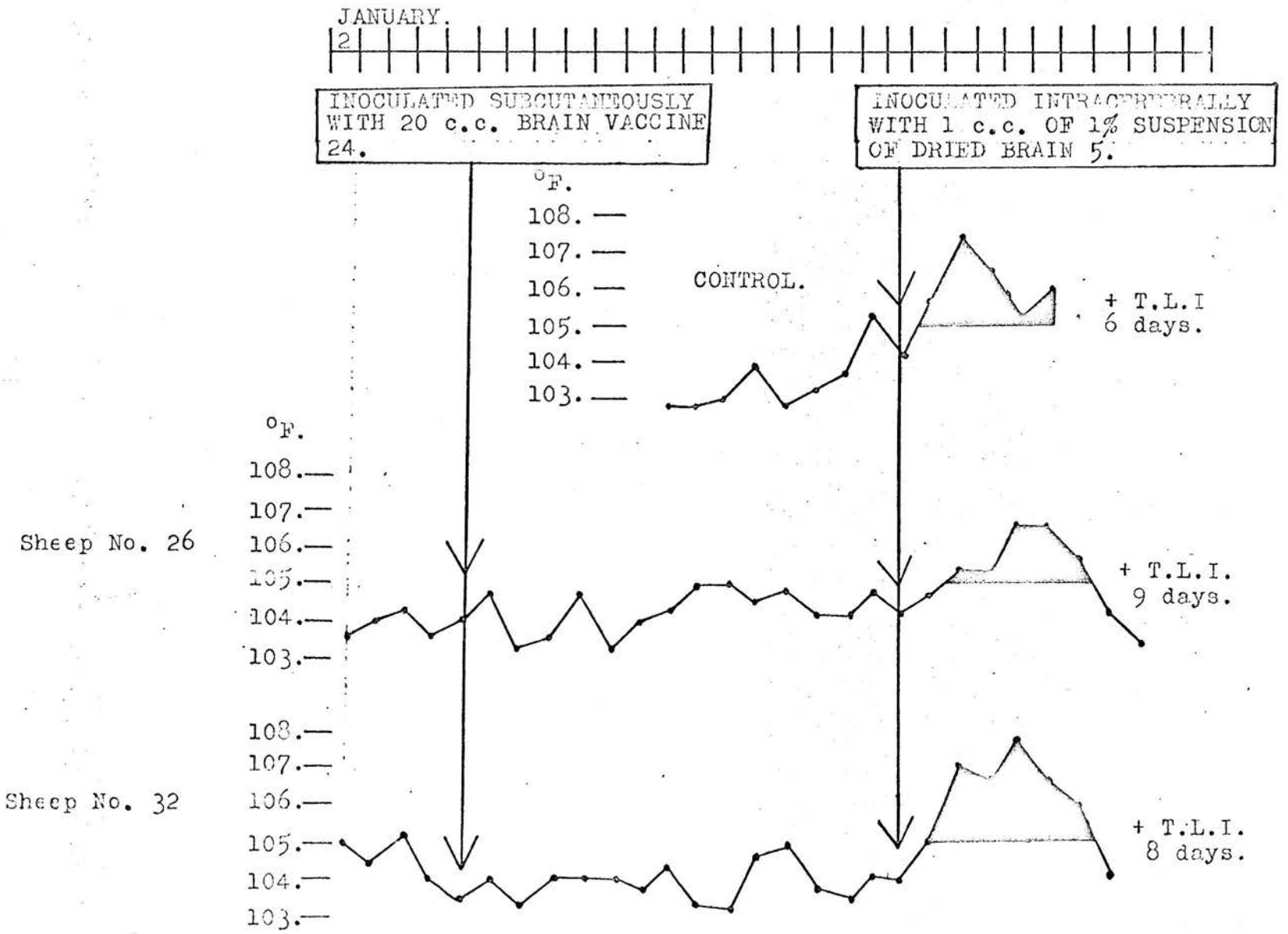

JANUARY.
2
INOCULATED SUBCUTANEOUSLY WITH 20 c.c. BRAIN VACCINE 24.
INOCULATED INTRACEREBRALLY WITH 1 c.c. OF 1% SUSPENSION OF DRIED BRAIN 5.
°F.
108.
107.
106.
105.
104.
103.
CONTROL.
+ T.L.I 6 days.
Sheep No. 26
+ T.L.I. 9 days.
Sheep No. 32
+ T.L.I. 8 days.

Figure XIII

Systemic immunity following one inoculation of dead vaccine subcutaneously.

Figure XIII

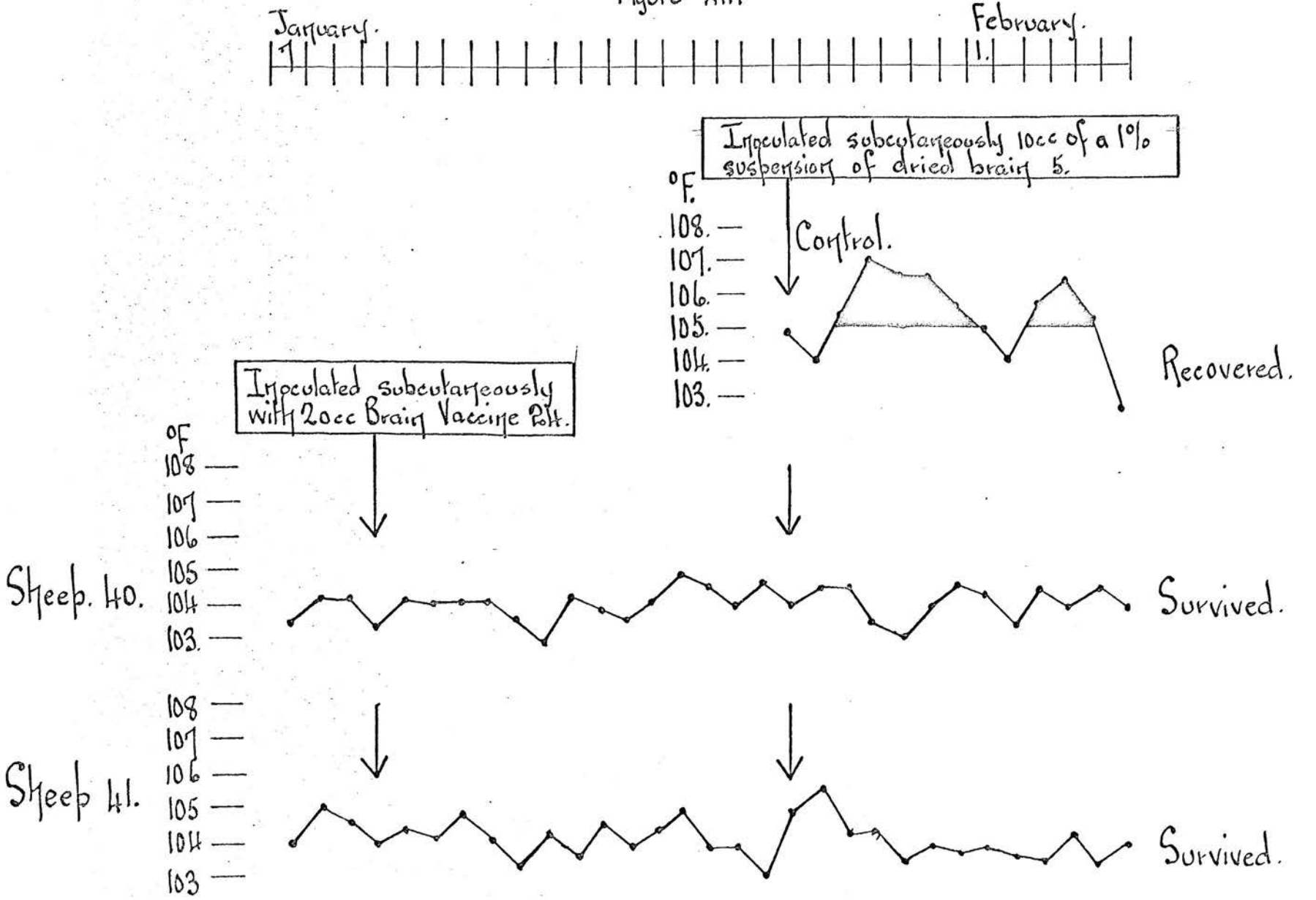

January.
1.
February.
1.
Inoculated subcutaneously 10cc of a 1% suspension of dried brain 5.
°F.
108.
107.
106.
105.
104.
103.
Control.
Recovered.
Inoculated subcutaneously with 20cc Brain Vaccine 24.
°F
108
107
106
105
104
103
Sheep. 40.
Survived.
108
107
106
105
104
103
Sheep 41.
Survived.

Figure XIV

No increase of immunity of central nervous system when living virus is inoculated subcutaneously following dead vaccine subcutaneously.

Figure XIV.

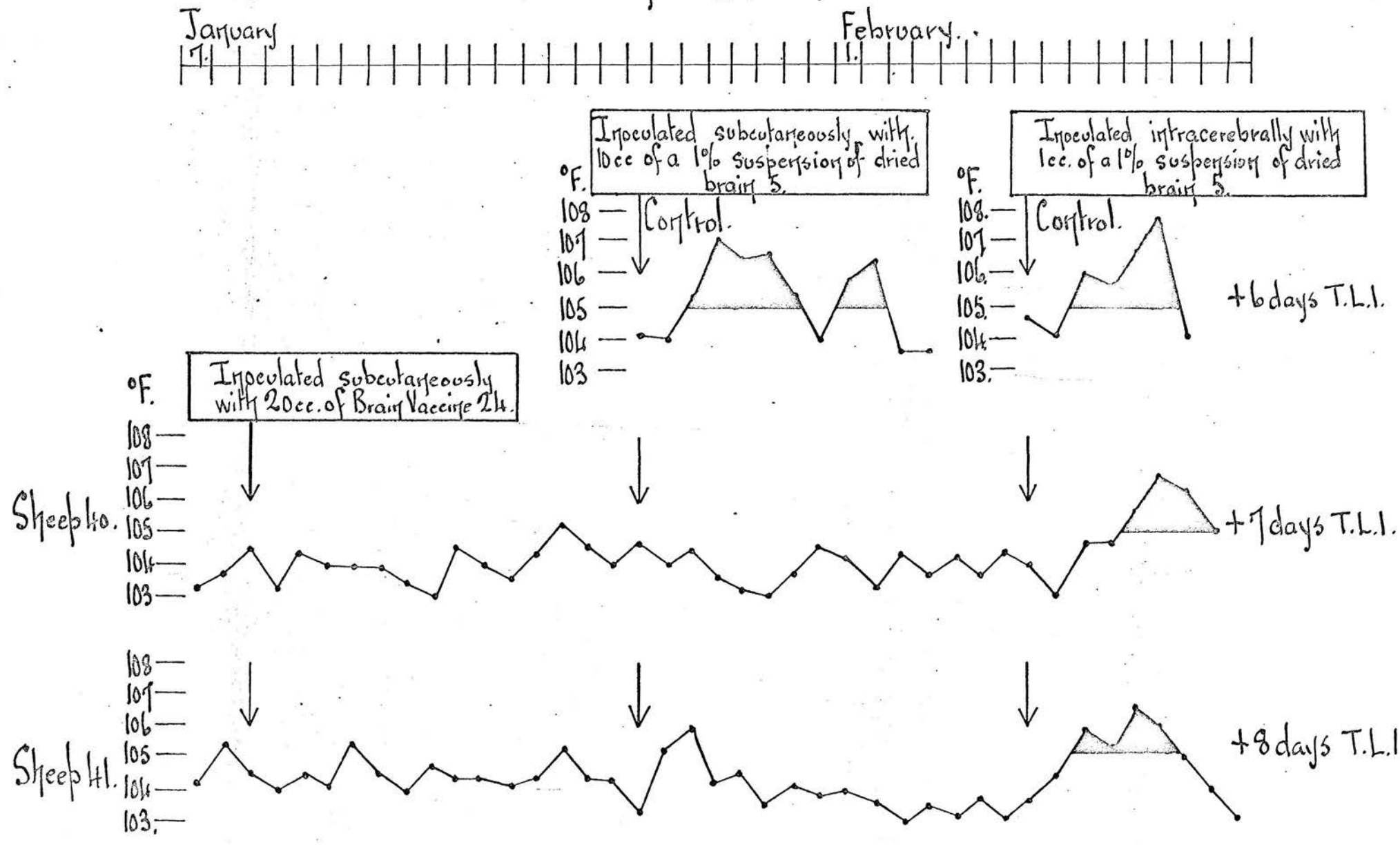

January
1.
February
1.
Inoculated subcutaneously with 10cc of a 1% suspension of dried brain 5.
Control.
Inoculated intracerebrally with 1cc. of a 1% suspension of dried brain 5.
Control.
+6 days T.L.I.
°F.
108
107
106
105
104
103
Inoculated subcutaneously with 20cc. of Brain Vaccine 24.
Sheep No.
+7 days T.L.I.
Sheep No.
+8 days T.L.I.

Figure XV

Some degree of immunity of the central nervous system following two doses of dead vaccine subcutaneously.

Figure XV.

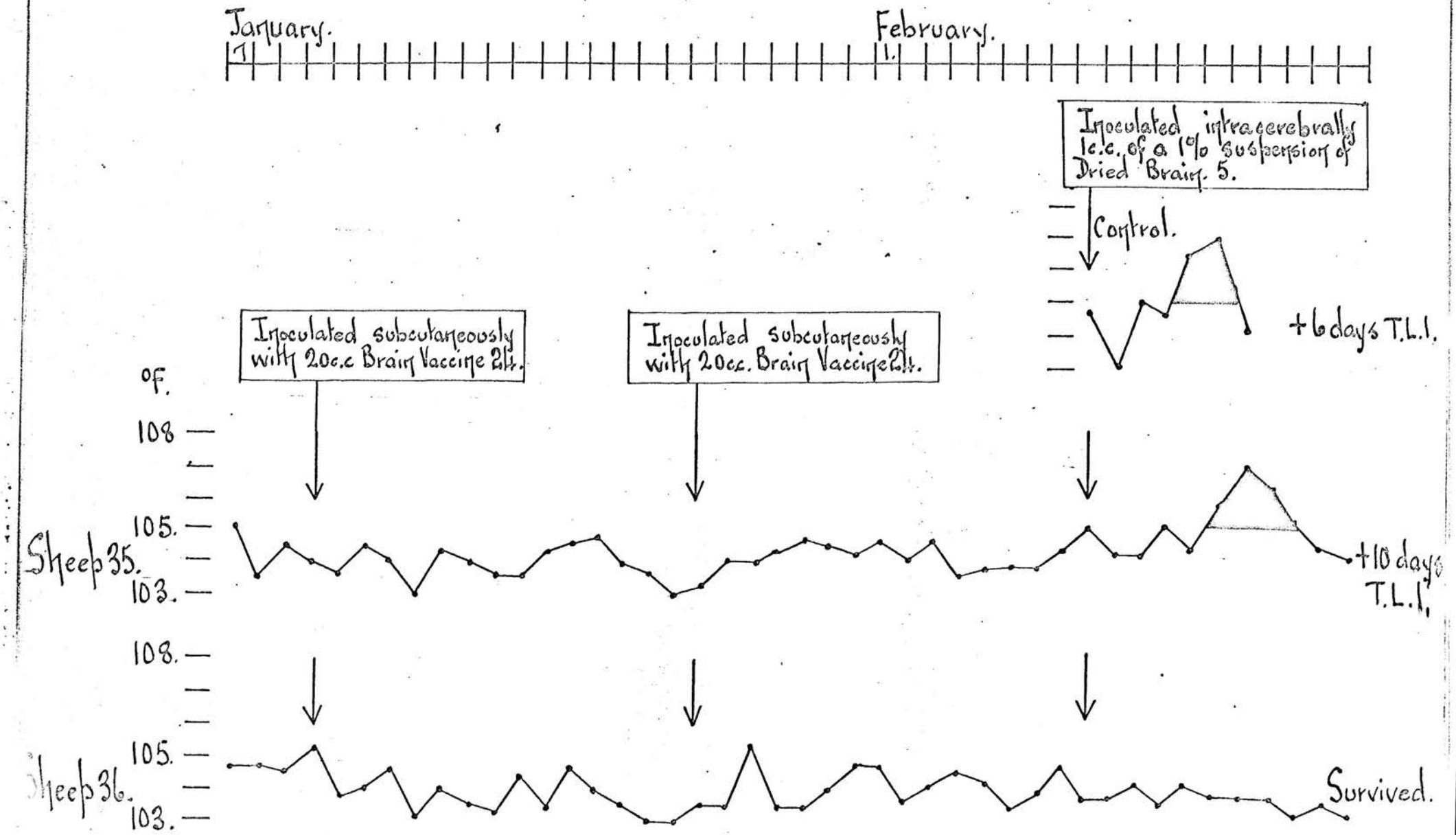

January.
1.
February.
1.
Inoculated intracerebrally 1c.c. of a 1% suspension of Dried Brain. 5.
Control.
+6 days T.L.I.
Inoculated subcutaneously with 20c.c Brain Vaccine 2½.
Inoculated subcutaneously with 20cc. Brain Vaccine 2½.
°F.
108
105.
103.
Sheep 35.
+10 days T.L.I.
108.
105.
103.
Sheep 36.
Survived.

Figure. XVI.

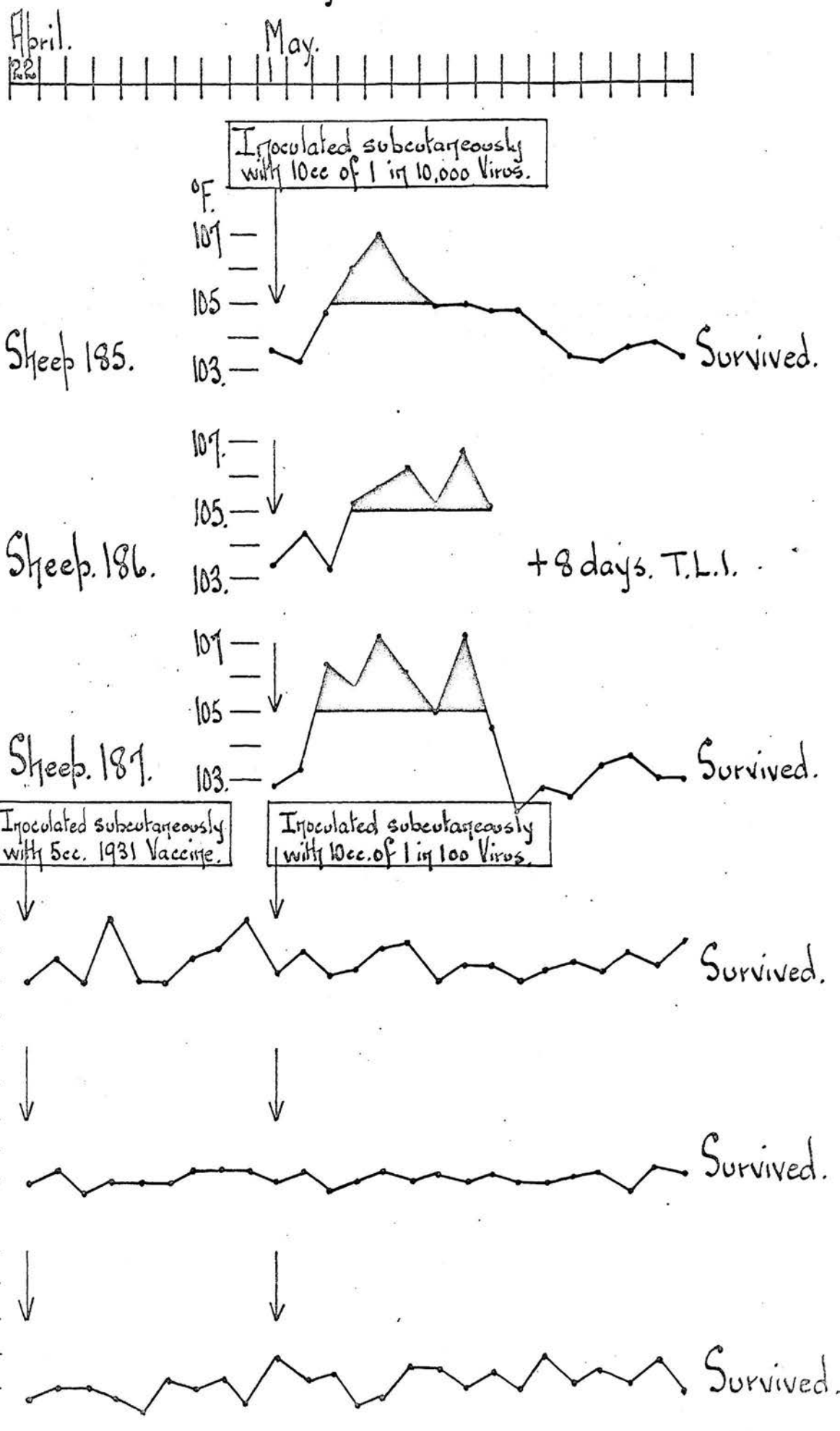

April.
22
May.
Inoculated subcutaneously with 10cc of 1 in 10,000 Virus.
°F.
107
105
103.
Sheep 185.
Survived.
107.
105.
103.
Sheep. 186.
+8 days. T.L.I.
107
105
103.
Sheep. 187.
Survived.
°F.
Inoculated subcutaneously with 5cc. 1931 Vaccine.
Inoculated subcutaneously with 10cc. of 1 in 100 Virus.
107.
105
103
Survived.
107.
105.
103.
Survived.
107
105.
103.
Survived.

Figure XVII

Result of Field Trial of Louping-ill Prophylactic Vaccine - 1931

Solid black = Percentage total death rate from all causes in vaccinated hoggs.

Shaded black = Percentage total death rate from all causes in non-vaccinated hoggs.

Taken from
Veterinary Record, XIV, 1, January 1934.

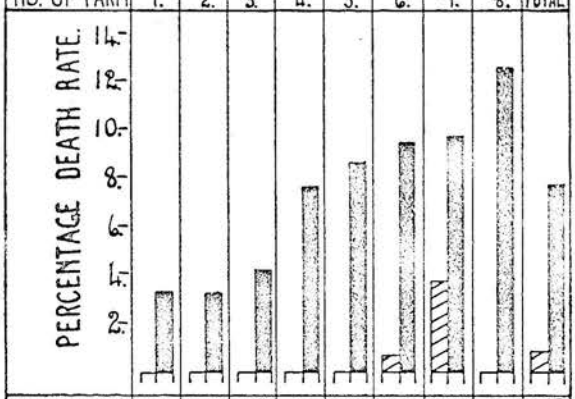
"The Control of Certain Diseases of Sheep"

by

W. S. Gordon.

Figure XVII.

LOUPING-ILL VACCINE FTELD TEST 1931.



NO. OF FARM	1.	2.	3.	4.	5.	6.	7.	8.	TOTAL
VACCINATED	25	40.	30.	90.	21.	165.	54.	30.	455
CASES OF L.I.	0.	0.	0.	0.	0.	1.	2.	0.	3.
CONTROLS.	30.	30.	24	91.	23.	107.	52.	24.	381.
CASES OF L.I.	1.	1.	1.	7.	2.	10.	5.	3.	30.

Figure XVIII

Immunising Value of Louping-ill Prophylactic Brain and Cord Vaccine - 1932.

Figure XVIII.

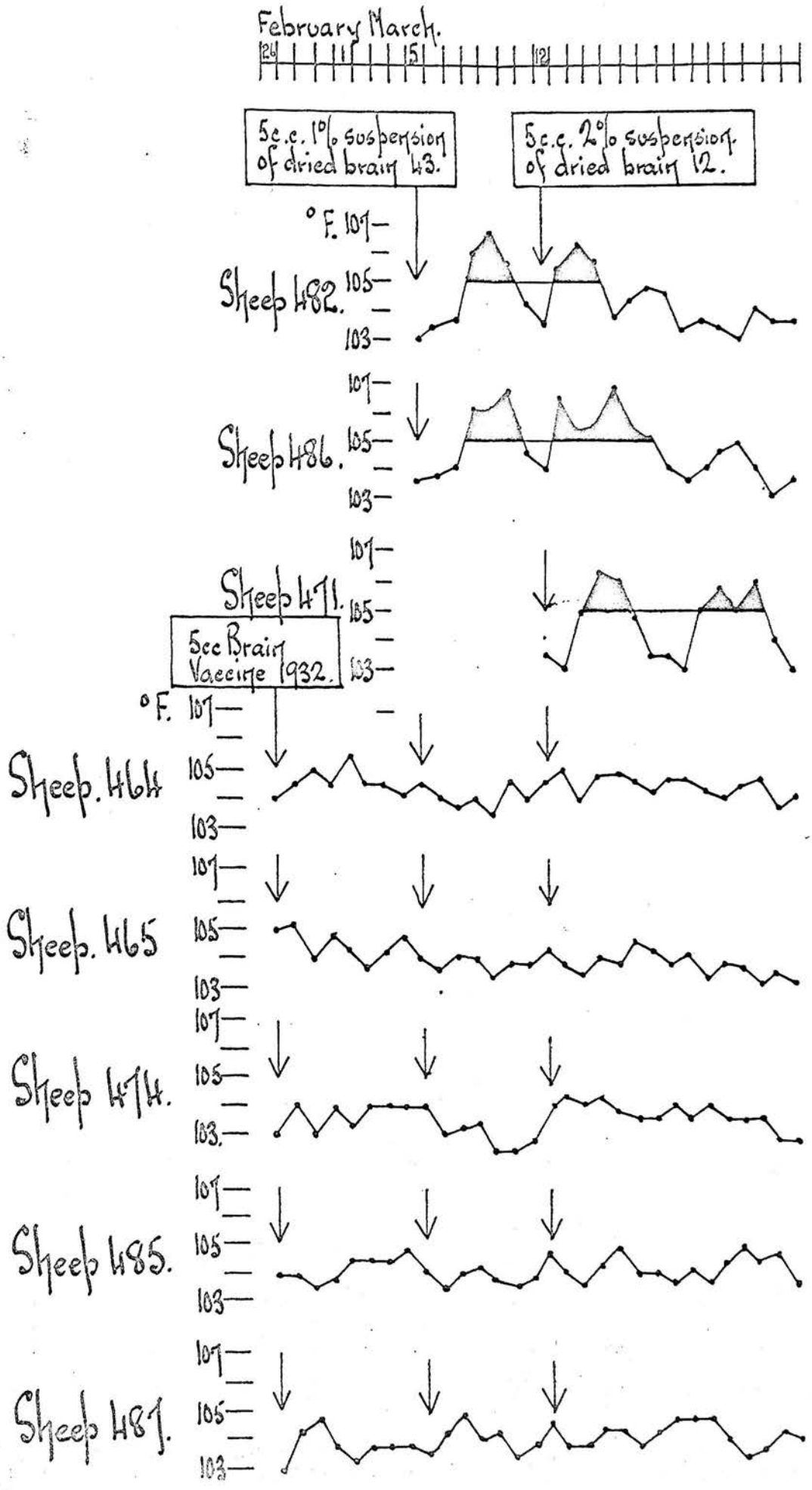


Figure XIX

Immunising Value of Louping-ill Prophylactic Spleen Vaccine - 1932

Figure XIX.

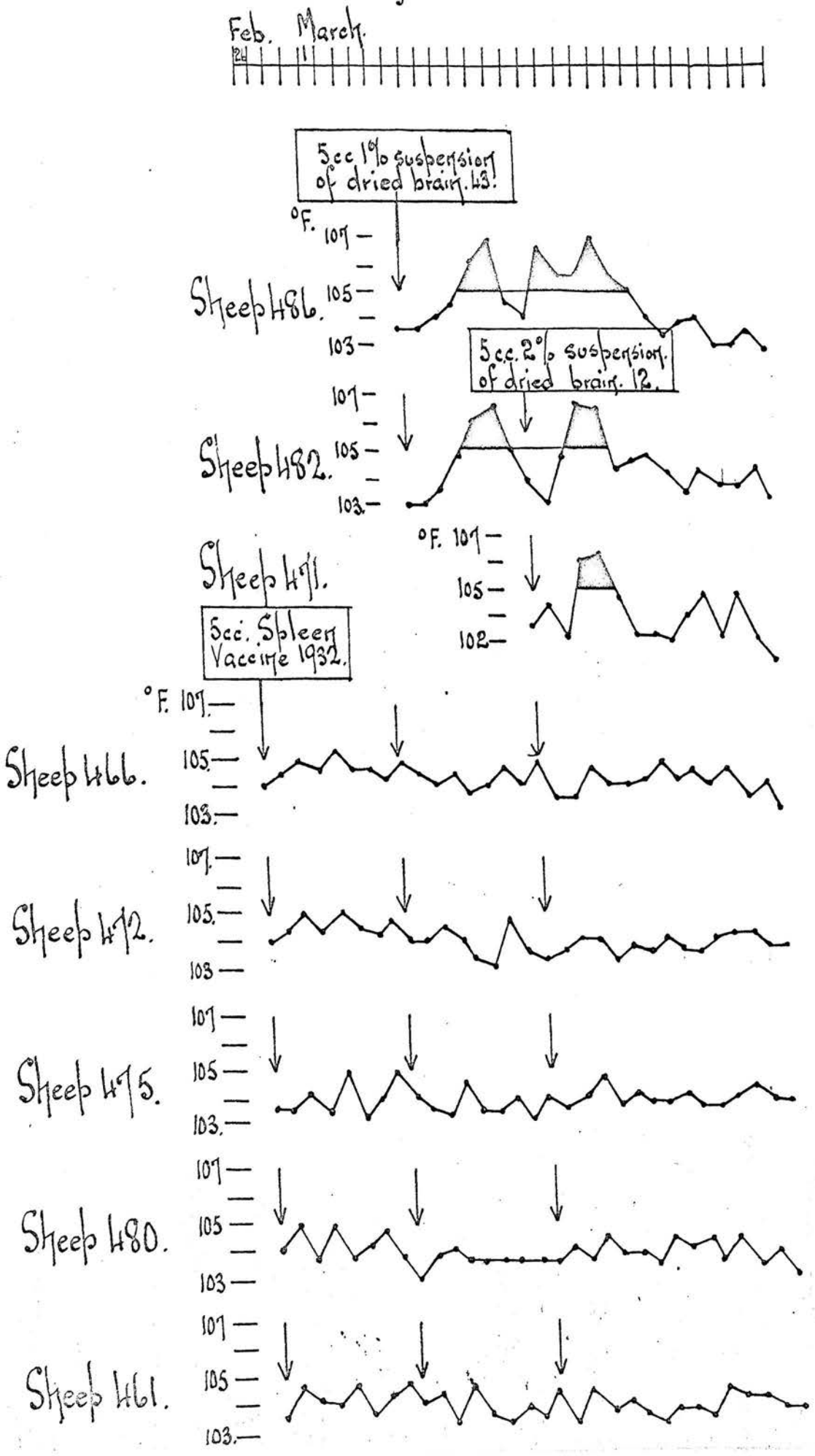

Feb.
March.
24
1
5cc 1% suspension of dried brain. 43.
°F.
107
105
103
Sheep 486.
5cc. 2% suspension of dried brain. 12.
Sheep 482.
Sheep 471.
°F. 107
105
102
5cc. Spleen Vaccine 1932.
Sheep 466.
Sheep 472.
Sheep 475.
Sheep 480.
Sheep 461.

Figure XX

Result of Field Trial of Louping-ill Prophylactic Vaccine - 1932 for the Prevention of Louping-ill in Hoggs.

Solid black = Percentage total death rate from all causes in vaccinated hoggs.

Shaded black = Percentage total death rate from all causes in non-vaccinated hoggs.

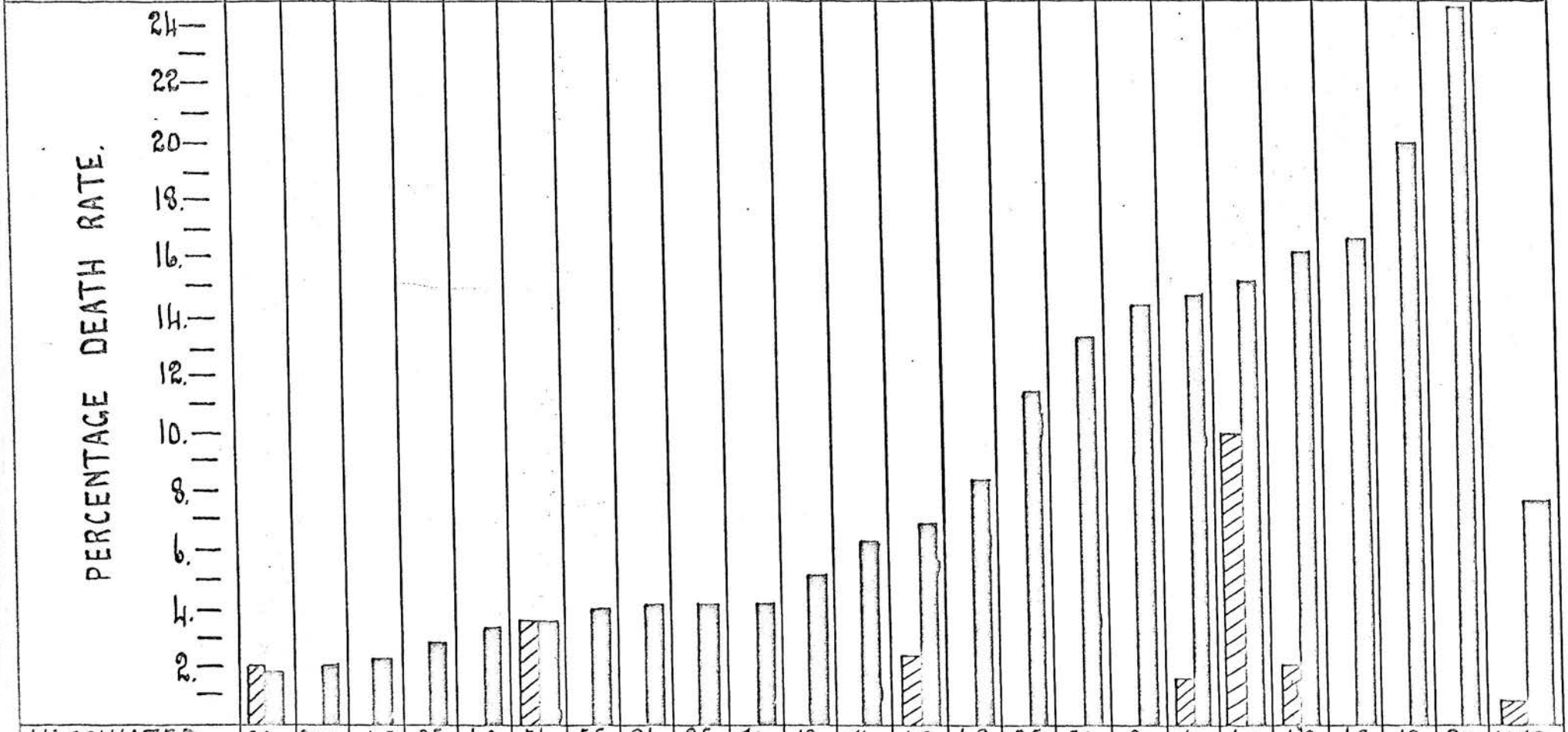
Taken from
Veterinary Record, XIV, 1, January 1934

"The Control of Certain Diseases of Sheep"

by

W. S. Gordon.

Figure xx.



FARM No.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16	17.	18.	19	20.	21	22	23.	TOTAL
VACCINATED.	50.	200.	45.	35.	60.	56.	55.	26	35.	70.	19.	76.	43.	48.	35.	50.	9.	70.	40.	49.	68.	13.	20.	1172.
DEATHS.	1.	0.	0.	0.	0.	2.	0.	0	0.	0.	0.	0.	1.	0.	0	0.	0.	1.	4.	1.	0	0	0.	10.
CONTROLS.	59.	200.	45.	35.	60.	56	50.	24	24.	94.	19.	94.	43.	48.	35	61.	7.	68.	40.	50.	67.	10	20.	1208.
DEATHS.	1.	4.	1.	1.	2.	2.	2.	1.	1.	4.	1.	6.	3.	4.	4	8.	1.	10.	6.	8.	11.	2.	5.	88.

Figure XXI

Result of Field Trial of Louping-ill Prophylactic Vaccine - 1932 for the Prevention of Louping-ill in Lambs

Solid black = Percentage total death rate from all causes in vaccinated lambs.

Shaded black = Percentage total death rate from all causes in non-vaccinated lambs.

Taken from

Veterinary Record, XIV, 1, January 1934.

"The Control of Certain Diseases of Sheep"

by

W. S. Gordon.

Figure XXI.

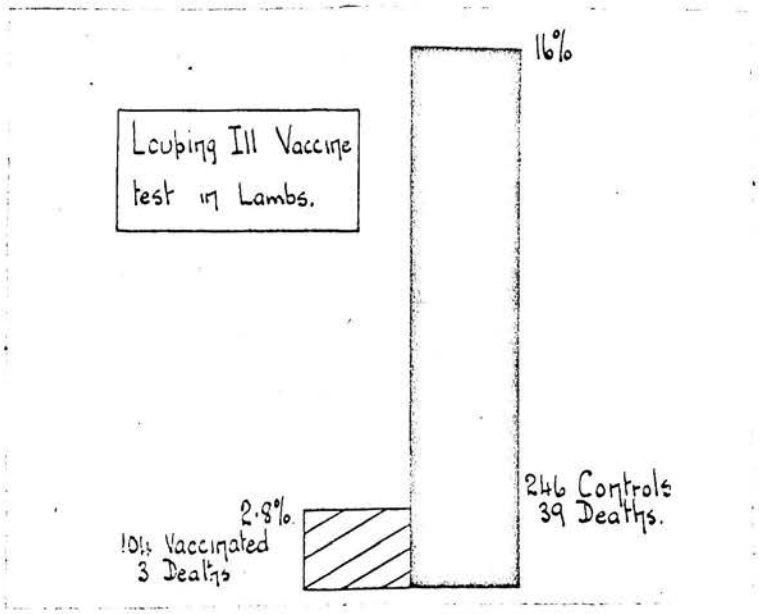

Louping Ill Vaccine
test in Lambs.
16%
246 Controls
39 Deaths.
2.9%
101 Vaccinated
3 Deaths

Figure XXII

Result of Field Trial of Louping-ill Prophylactic Vaccine - 1933 for the Prevention of Louping-ill in Hoggs

Solid red = Percentage death rate from louping-ill in vaccinated hoggs.

Shaded red = Percentage total death rate from all causes in vaccinated hoggs.

Solid black = Percentage death rate from louping-ill in non-vaccinated hoggs.

Shaded black = Percentage total death rate in non-vaccinated hoggs.

Figure XXIII

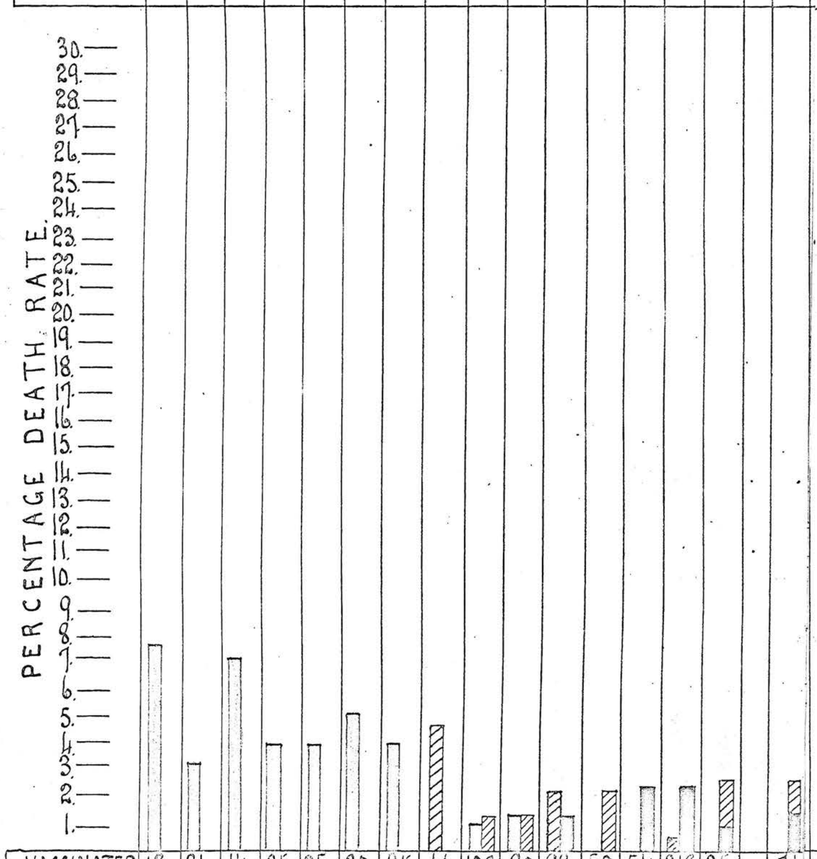
Result of Field Trial of Louping-ill Prophylactic Vaccine - 1933 for the Prevention of Louping-ill in Lambs

Solid red = Percentage death rate from louping-ill in vaccinated lambs.

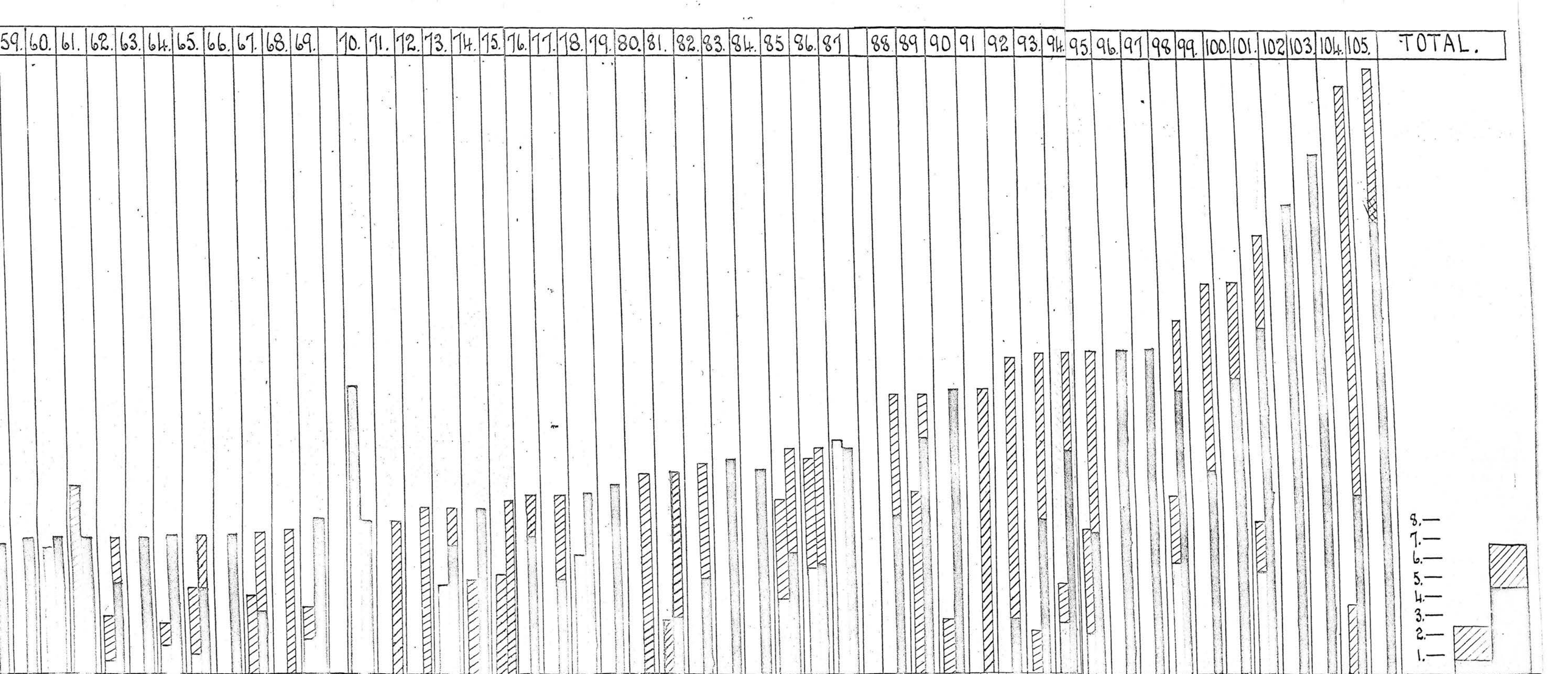
Shaded red = Percentage total death rate from louping-ill in non-vaccinated lambs..

Solid black = Percentage death rate from louping-ill in non-vaccinated lambs.

Shaded black = Percentage total death rate in non-vaccinated lambs.

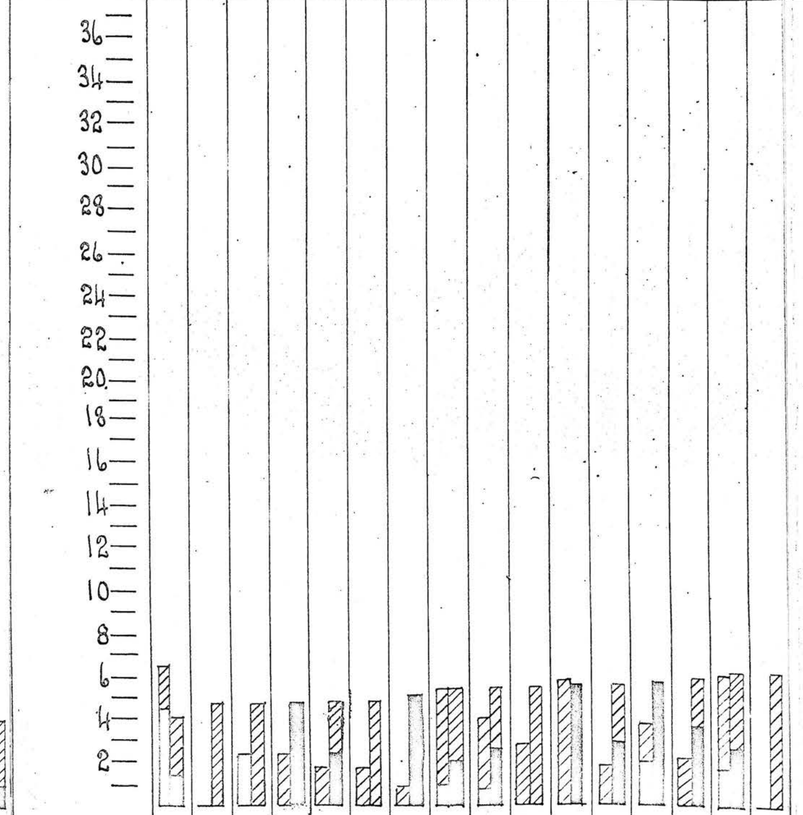


FARM No.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31	32.	33.	34.	35.	36.	38.	39.	40	41.	42.	43.	44.	45.	46.	47.	48.	49.	50.	51.	52.	53.	54	55	56	57.	58.	59.	60.
VACCINATED	13	31	14	25	25	20	25	66	108	80	93	50	54	218	25	71	10	64	30	29	27	49	105	120	80	25	23	75	100	23	99	65	90	68	25	22	60	83	41	56	60	50	73	18	94	35	18	19	50	35	17	16	102	50	105	63	48	48	16
DEATHS. LOUPING-ILL	1	1	1	1	1	1	1	0	1	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	2	0	0	0	1	0	0	0	0	1	0	0	0	3	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	4	0	0	0	0
OTHER CAUSES	0	0	0	0	0	0	0	3	0	0	2	0	0	1	0	0	0	3	0	0	0	0	2	3	1	0	2	0	0	0	1	2	2	0	0	0	2	0	0	0	0	0	1	0	2	1	0	0	0	0	0	0	0	3	0	2	0	0	0	0
TOTAL.	1	1	1	1	1	1	1	3	1	1	2	0	0	1	0	0	0	4	0	1	0	0	2	3	3	0	2	0	1	0	1	2	2	1	0	0	2	3	0	0	0	0	1	1	2	1	0	1	0	0	0	0	0	3	4	2	0	0	0	0
NON VACCINATED.	12	32	14	25	25	18	25	55	78	78	71	48	43	253	120	79	36	64	31	28	26	52	105	124	75	25	24	71	120	22	133	65	86	63	42	21	6	82	40	60	59	59	78	19	92	36	18	18	70	35	17	17	102	65	80	64	45	46	15
DEATHS. LOUPING-ILL	0	0	0	0	0	0	0	0	0	0	1	0	1	6	1	1	0	1	1	1	1	2	0	2	2	1	1	3	5	1	3	3	4	3	2	0	3	4	1	2	3	0	0	1	4	1	0	0	4	2	1	0	6	4	5	3	3	3	1
DEATHS. OTHER CAUSES	0	0	0	0	0	0	0	0	1	1	0	1	0	0	2	1	1	1	0	0	0	0	4	3	1	0	0	0	0	0	3	0	0	0	0	1	0	0	1	1	3	3	4	0	1	1	1	1	0	0	0	1	0	0	0	1	0	0	0
DEATHS. TOTAL.	0	0	0	0	0	0	0	0	1	1	1	1	1	6	3	2	1	2	1	1	1	2	4	5	3	1	1	3	5	1	6	3	4	3	2	1	3	4	1	3	3	3	4	1	5	2	1	1	4	2	1	1	6	4	5	3	3	3	1

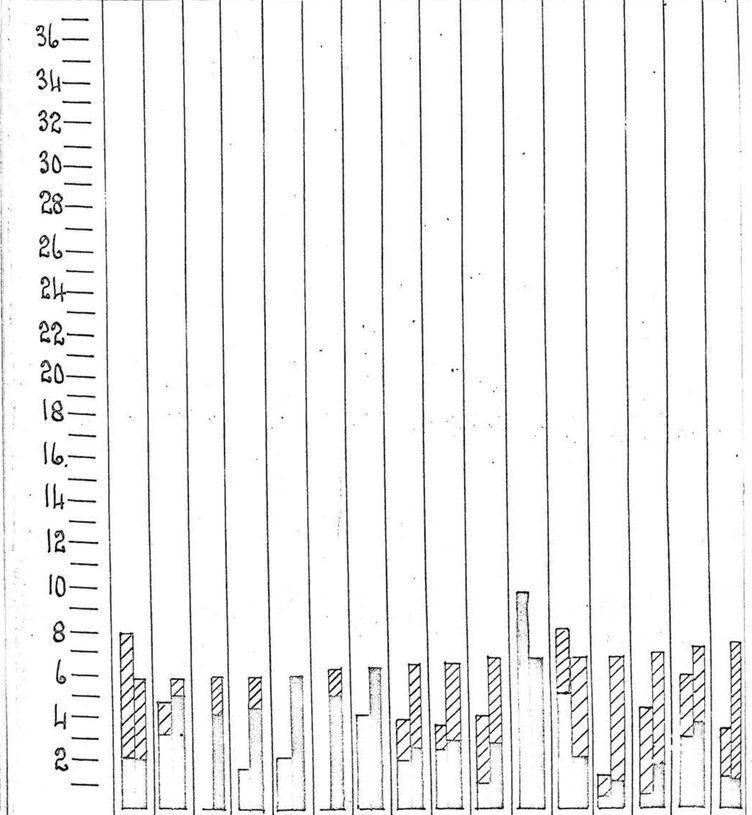


59.	60.	61.	62.	63.	64.	65.	66.	67.	68.	69.	70.	71.	72.	73.	74.	75.	76.	77.	78.	79.	80.	81.	82.	83.	84.	85.	86.	87.	88.	89.	90.	91.	92.	93.	94.	95.	96.	97.	98.	99.	100.	101.	102.	103.	104.	105.	TOTAL.
3	16	16	21	163	40	89	120	40	105	15	65	71	27	14	46	22	61	55	27	17	21	24	40	31	18	9	56	56	17	50	43	40	11	42	52	45	110	18	12	55	20	20	40	34	12	34	14	508 H.
0	0	1	2	1	0	1	1	0	0	0	1	1	0	0	2	0	0	0	0	1	0	0	0	0	0	0	2	2	2	0	0	0	0	0	0	1	2	0	0	3	0	0	2	0	0	0	0	50.
0	0	0	0	3	0	1	4	0	4	0	1	0	0	0	0	1	3	0	0	0	0	0	1	0	0	0	3	3	0	0	4	1	0	0	1	1	6	0	0	2	0	0	1	0	0	1	0	74.
0	0	1	2	4	0	2	5	0	4	0	2	1	0	0	2	1	3	0	0	1	0	0	1	0	0	0	5	6	2	0	4	1	0	0	1	2	8	0	0	5	0	0	3	0	0	1	0	124.
6	15	15	15	163	30	73	146	29	99	14	65	13	26	12	48	24	34	45	22	11	32	10	40	67	19	10	99	90	18	51	43	35	14	40	53	46	115	18	12	56	10	20	40	33	15	33	13	5429.
3	1	1	1	7	2	5	6	2	3	0	5	1	0	0	3	2	0	3	1	1	3	0	1	3	2	1	6	5	2	4	5	5	0	1	4	5	8	3	2	8	1	3	7	8	4	3	3	237.
0	0	0	0	4	0	0	4	0	4	1	0	0	2	1	1	0	3	1	1	0	0	1	3	4	0	0	5	5	0	3	1	0	2	5	4	2	11	0	0	2	1	1	2	0	0	1	1	117.
3	1	1	1	11	2	5	10	2	7	1	5	1	2	1	4	0	3	4	2	1	3	1	4	7	2	1	11	10	2	7	6	5	2	6	8	7	19	3	2	10	2	4	9	8	4	10.	4.	354

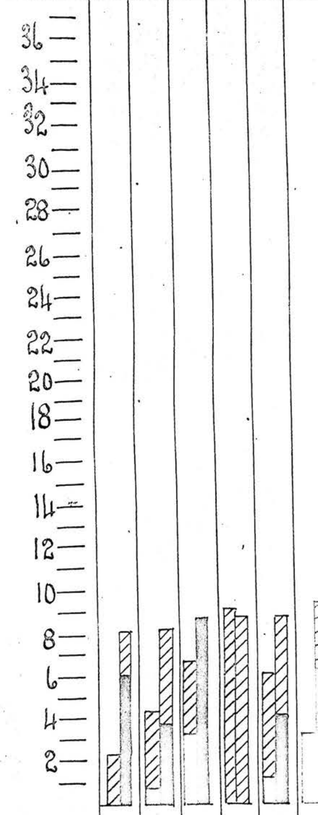
FARM No.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.
VACCINATED.	75	57	190	198	66	45	129	31	35	28	54	99	363
DEATHS LOUPING-ILL.	0	1	0	0	5	1	0	2	0	2	1	2	0
DEATHS OTHER CAUSES.	2	0	2	1	0	1	0	1	0	1	1	1	11
DEATHS TOTAL.	2	1	2	1	5	2	0	3	0	3	2	3	11
NONVACCINATED	87	83	206	124	114	77	101	33	160	28	54	131	410
DEATHS LOUPING-ILL.	1	1	3	1	1	6	0	1	4	1	2	2	4
DEATHS OTHER CAUSES.	0	0	0	1	2	2	3	0	1	0	0	3	12
DEATHS TOTAL.	1	1	3	2	3	2	3	1	5	1	2	5	16



	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.
	108	13	92	48	119	123	120	118	179	239	36	60	60	151	197	49
	5	0	2	0	0	0	0	1	1	0	0	0	1	0	3	0
	2	0	0	1	2	2	1	5	6	6	2	1	1	3	8	0
	7	0	2	1	2	2	1	6	7	6	2	1	2	3	11	0
	253	22	88	65	129	121	100	111	147	242	38	75	75	180	228	51
	4	0	0	3	3	0	5	2	4	0	2	2	4	6	5	0
	6	1	4	0	3	6	0	4	5	14	0	2	0	4	8	2
	10	1	4	3	6	6	5	6	9	14	2	4	1	10	13	2



	30.	31.	32.	33.	34.	35.	36.	37.	38.	39.	40.	41.	42.	43.	44.	45.
	138	124	50	190	45	33	46	50	160	98	30	155	227	160	160	140
	3	4	0	3	1	0	2	1	4	1	3	8	1	1	5	2
	8	2	0	0	0	0	0	1	2	3	0	5	2	4	5	3
	11	6	0	3	1	0	2	2	6	4	3	13	3	8	10	5
	203	202	50	200	49	240	46	119	135	102	29	185	221	170	160	154
	4	10	2	9	3	12	3	3	4	3	2	4	4	3	6	2
	8	2	1	3	0	3	0	5	5	4	0	9	12	9	6	10
	12	12	3	12	3	15	3	8	9	7	2	13	16	12	12	12



	46.	47.	48.	49.	50.	51.
	86	140	30	145	184	
	0	1	1	0	2	
	2	5	1	4	9	
	2	6	2	4	11	
	50	110	35	70	174	
	3	4	3	0	7	
	1	5	0	6	8	
	4	9	3	6	15	

49.	50.	51.	52.	53.	54.	55.	56	57.	58.	59	60.	61.
45	184	33	42	43	183	32	110	30	134	160	240	74
0	2	1	1	1	2	0	5	0	1	0	8	1
4	9	0	1	1	4	2	4	2	5	1	9	0
4	11	1	2	2	6	2	9	2	6	1	17	1
70	174	33	55	43	172	32	154	30	120	201	212	76
0	7	2	3	2	11	2	10	3	1	11	10	8
6	8	1	2	2	5	1	5	0	11	10	12	0
6	15	3	5	4	16	3	15	3	12	21	22	8

62.	63.	64.	65.	66.	67.	68.	69.	70.	71.	72.	73.	74.	75.	76.	77.
198	142	92	60	155	180	60	198	290	320	71	32	33	105	66	110
5	0	0	0	5	0	0	1	0	0	0	1	0	0	0	9
5	12	0	1	5	1	0	0	30	31	0	0	0	3	0	21
10	12	0	1	10	1	0	1	30	31	0	1	0	3	0	29
144	183	100	70	151	180	60	169	293	240	80	36	35	105	49	165
10	0	6	6	15	13	6	6	0	0	11	4	2	11	5	16
5	20	5	2	3	8	1	15	31	32	0	1	3	4	2	10
15	20	11	8	18	21	7	21	31	32	11	5	5	15	7	26

78.	79.	80	81.	82.	83.	84.	85.	86.	87	TOTAL.
48	73	37	76	87	78	115	43	40	57	9032.
3	0	3	3	5	1	3	0	0	0	128.
5	0	0	9	0	1	0	0	11	0	234.
8	0	3	12	5	2	3	0	11	0	412.
53	80	34	73	76	70	100	50	35	63	10,137.
2	14	6	2	17	16	28	15	0	21	444.
1	0	0	12	0	0	0	0	13	3	412.
9	14	6	14	17	16	28	15	13	24	856.